U.S. DEPARTMENT OF COMMERCE
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PB-261 006

SUBSTITUTE CHEMICAL PROGRAM
THE FIRST YEAR OF PROGRESS
PROCEEDINGS OF A SYMPOSIUM
VOLUME III. ECOSYSTEMS/MODELING WORKSHOP

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C.

1975

BIBLIOGRAPHIC DATA SHEET

1. Report No. EPA-540/6-76-015

2.

3. Recipient's Accession No.

4. Title and Subtitle Substitute Chemical Program - The First Year of Progress . Proceedings of a Symposium
Volume 3 -- Ecosystems/Modeling Workshop

5. Report Date July 30 - August 1, 1975

6.

7. Author(s)

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
U.S. Environmental Protection Agency, Office of Besticide Programs and Office of Research and Development, Washington, D.C. 20460

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Address
SAME AS BOX 9

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstracts

Volume 3 covers the Ecosystems/Modeling Workshop of the Symposium. Fourteen papers are published, on such topics as: review of the program status and direction of future NERL programs; terrestrial in-house systems; terrestrial microcosm development; effects of mirex on the predator-prey interactions in an experimental estuarine ecosystem; effects of mirex on the burrowing activity of the lugworm (Arenicola cristata); status report on fresh water ecosystem methodology development; research plan for whole systems model -- design, validation, and integration between terrestrial and aquatic environment; mathematical modeling of pesticide fate: effects of pesticide use patterns on the incidence of plant disease and on patterns of pesticide degradation; benchmark chemistry; development of rapid screening techniques for herbicide phytotoxicity; analytical methods development.

17. Key Words and Document Analysis. 17a. Descriptors

ecosystems
chemicals
microcosm development
mirex
predator-prey interactions
estuarine ecosystem
lugworm
fresh water ecosystem
research
pesticide fate
mathematical modeling
pesticide use patterns
plant disease
pesticide degradation
rapid screening techniques
herbicide phytotoxicity
microcosm methodology development
analytical methods development

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group

18. Availability Statement

19. Security Class (This Report) UNCLASSIFIED

20. Security Class (This Page) UNCLASSIFIED

21. No. of Pages 154

22. Price 6.75-3.00

FORM NTIS-35 (REV. 10-73) ENDORSED BY ANSI AND UNESCO. THIS FORM MAY BE REPRODUCED USCOMM-DC 8265-P74

PB 261 006

[illegible] FIRST YEAR OF PROGRESS

PROCEEDINGS OF A SYMPOSIUM

Volume II

Assessment/Modeling Workshop

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SUBSTITUTE CHEMICAL PROGRAM - THE FIRST YEAR OF PROGRESS

PROCEEDINGS OF A SYMPOSIUM

Volume III

Ecosystems/Modeling Workshop

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SHERATON-FREDERICKSBURG MOTOR INN
FREDERICKSBURG, VIRGINIA

JULY 30 - AUGUST 1, 1975

The Proceedings of the SUBSTITUTE CHEMICAL PROGRAM -- THE FIRST YEAR OF PROGRESS are published in four volumes. Volume I contains speeches and discussion from the Plenary Session, the agenda, and lists of participants and speakers. Volumes II, III, and IV cover the Toxicological Methods and Genetic Effects Workshop, the Ecosystems/ Modeling Workshop, and the Chemical Methods Workshop, respectively.

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ECOSYSTEMS/MODELING WORKSHOP
OPENING REMARKS

Dr. John L. Buckley*

There are some detailed models which seem to be reasonably good for what happens in local areas. There are some conceptual models that seem to me to be highly useful. I guess I feel somewhat better about it now than I did a year ago. The tools available to us through the activities of many of you in this room and others have really provided some additional data and some additional methods that can be used for looking at this question of what happens to this particular set of chemicals in the environment.

I'd make one other point and I make it because of the position that I was in last night. I was there to talk about the Substitute Chemical Program and I did for about 15 minutes out of a two-hour program. The rest of the session occasionally touched on the Substitute Chemical Program, but more than that, it touched on why EPA had made some of the decisions that it had made.

I was reminded that the quality of the decisions that get made isn't going to be any better than the quality of the information available at the time the decisions were made. I suppose that is an obvious statement. The other half of the statement is that availability of good information doesn't assure that good decisions will be made. I don't mean to put down any individual decision but I note that the two aspects are separable and at the same time intricately related. It seems to me that our concern here should be with how we can improve the quality of the information that can be used in making the decisions.

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*Acting Deputy Assistant Administrator for Program Integration, EPA

REVIEW OF WHOLE PROGRAM STATUS AND DIRECTION OF FUTURE NERL PROGRAMS

Dr. Norman R. Glass*

Briefly, I'd like to make just a couple of very quick comments and then move more directly into the program in some depth. The first comment that I'd like to make I think most of you are aware of but for those who aren't, let me restate it. This Program was funded in May of 1974, a scant year and a half ago, and what that really means is that there are some portions of the Program that are very new and some portions that are somewhat older.

There are three primary components to the Program. These three objectives are to develop a microcosm methodology which can be used as a pre-market screening device for proposed substitute chemicals; second, through the development of the microcosm methodology we hope to be able to get at some of the basic ecological processes which may be affected by certain of these substitute chemicals; and third, we hope to be able to integrate some of these data and information by the use of modeling techniques.

To carry out those three major program objectives there are four organizational components that have been involved. The four components correspond roughly to the four research laboratories within the EPA's Office of Research and Development that have expertise bearing on this problem.

First there's the Corvallis Environmental Research Laboratory of which I am a part. We are responsible for coordination and overall project management and direction and also for the terrestrial research part of the Program and modeling. The Gulf Breeze Environmental Research Laboratory is the second major component of the Program and their responsibility is primarily in the marine and estuary portions. Third, the Duluth Laboratory

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* National Ecological Research Laboratory, Corvallis, Oregon

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is responsible for fresh water toxicology and fresh water ecological research. Fourth, the Athens Laboratory is responsible for some work in modeling as well as work in fresh water ecology.

In addition to the in-house Program, there is also an extramural portion conducted through contract, grant, and interagency agreements, and these components supplement the in-house part of the Program.

We have for example, Dr. Sanborn from the University of Illinois who will be discussing part of the work that he and Dr. Metcalf have been doing there under contract to the Corvallis Lab. Dr. Isensee from USDA in Beltsville, Maryland is going to discuss the results of his and Dr. Kearney's work on microcosms. Dr. Goodman from Michigan State is going to talk to us about the progress of his grant which was funded one month ago, so this will be a short story. Also Pete Schoor from Gulf Breeze may choose to cover some portions of their extramural program in addition to his own in-house work. Last, but not least, we have three representatives of the Criteria and Evaluation Division who will present the results of the contracts they've been overseeing for the past year.

In summary, the Program is broken down into three components in terms of medium into marine, fresh water, and terrestrial. It's broken down into three objectives: the development of microcosms, the use of microcosms to improve our understanding of process, and the use of models for integration. This Program is being discharged by the four laboratories which I have just identified and by their corresponding extramural contractors.

Finally, during the course of the past year to year and a half, in addition to discharging the research program per se, we've also reviewed 25 or 26 chemicals that have come from OPP under the Scientific and Mini-Economic Review process.

PROGRESS AND STATUS REPORT ON TERRESTRIAL IN-HOUSE SYSTEM*

Dr. James W. Gillett**

The Substitute Chemical Program (SCP) is charged by the Congress with developing procedures for determining the safety of materials registered for uses for which other agents have been limited or deregistered. The National Ecological Research Laboratory is carrying out a program of research on the disposition and effects of these pesticides in laboratory microcosms in terrestrial/atmospheric, estuarine/marine, and freshwater systems. A conceptual model indicating the interrrelationship within and between these media has been produced (2). The need to know or project accurately potential effects of chemicals deliberately or accidentally added to the environment forms the basis for the development of laboratory ecosystem simulators. Controlled environmental parameters (light, temperature, humidity, air and water movements) and defined biotic and abiotic elements are combined to form a laboratory system representing processes typical of the real world. Parallel development of mathematical models and simulators permits interpretation and extrapolation of effects. Laboratory microcosms represent an intermediate level of investigation between the "test-tube" or single animal toxicology test and field studies of natural ecosystems.

Historical Background

During the late 1960s Dr. Robert Metcalf of the University of Illinois led a group of investigators (3) into developing a "farm pond" laboratory system (Figure 1a) that has been used to assess the environmental fate and, to a lesser extent, effects of over 100 pesticides and other hazardous chemicals. In spite of a number of deficiencies inherent in trying to simulate anything as complex as a natural

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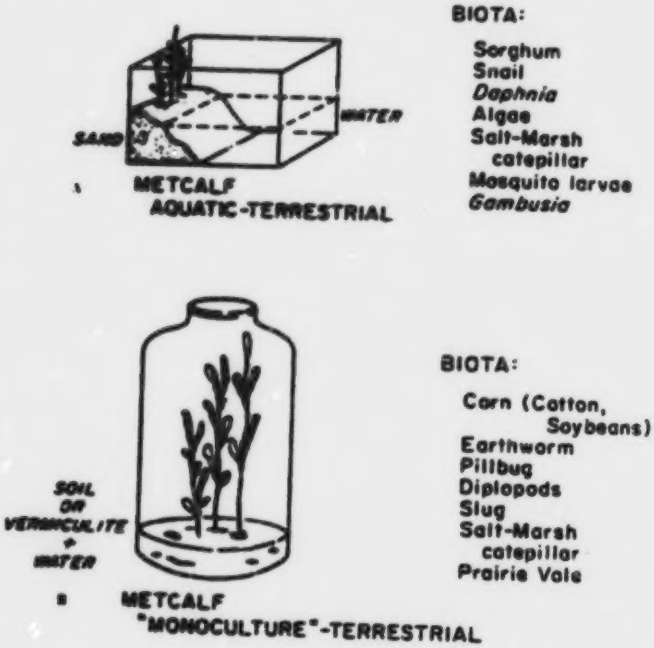
*Co-author is Jay D. Gile

**National Ecological Research Laboratory, Corvallis Environmental Research Laboratory, Office of Research and Development, EPA, Corvallis, Oregon ecosystem, these aquatic/terrestrial microcosms have demonstrated their utility and basic validity by providing information on the disposition of chemicals in the biota and in the abiotic elements (water, "soil," and chamber. The data from experiments can be compressed into two indices of disposition: Ecological Magnification (E. M.), the ratio of the concentration in any biological element to the concentration in water or soil; and the Biodegradability Index (B. I.), the ratio of polar metabolites (water-soluble breakdown products) to the remaining chemical agent. For persistent chemicals (for which broad experience in the field provides a direct basis for comparison) the E. M. s are large (usually greater than 1000) and B. I. s are low (usually less than 0.5). Conversely, the E. M. s are low (100 or less) and B. I. s high (greater than 1) for a number of agents, including some which might be employed as replacements for the environmentally difficult, persistent pesticides.

As an element of the Substitute Chemical Program, NERL has continued EPA support of the work of Dr. Metcalf, who is now developing a terrestrial "monoculture" microcosm (Figure 1b) that can be related to major crop usage of such substitute chemicals. Thus far, this system (1) has been employed with aldrin, DDT, dyfonate, and methoxychlor. Accountability of material has been increased to about 65 to 75 percent and at least two specific research problems of major interest have been generated. In the first instance, Cole et al. (1) have quantified the phytotoxicity of aldrin/dieldrin to corn. Second, they have found a major and as yet unidentified metabolite of aldrin in corn. It is highly significant that microcosm research can develop new aspects for study even with an agent that has been used for a quarter century. Less spectacularly but of greater interest to the program, the tests to date with the "monoculture" terrestrial microcosm have shown the same patterns of residues and critical indices (B. I., E. M.) observed in both the "farm pond" system and the real world.

Figure 1

A. Metcalf Aquatic/Terrestrial Microcosm
B. Metcalf Terrestrial "Monoculture" Microcosm


BIOTA:
Sorghum
Snail
Daphnia
Algae
Salt-Marsh catepillar
Mosquito larvae
Gambusia
SAND
WATER
A METCALF AQUATIC-TERRESTRIAL
BIOTA:
Corn (Cotton, Soybeans)
Earthworm
Pillbug
Diplopods
Slug
Salt-Marsh catepillar
Prairie Vole
SOIL OR VERMICULITE + WATER
B METCALF "MONOCULTURE"-TERRESTRIAL

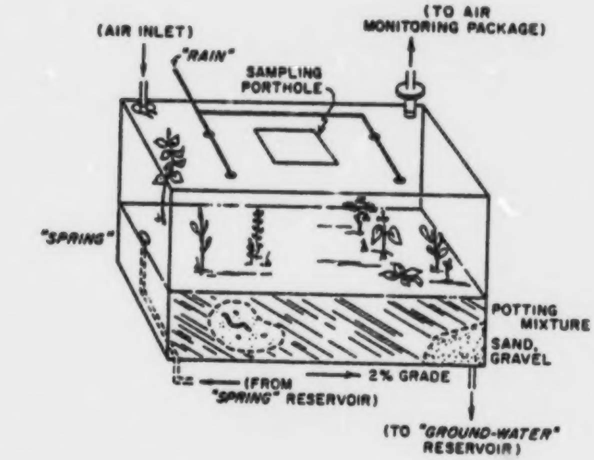
The NERL Terrestrial Microcosm

At the NERL facility in Corvallis, terrestrial microcosm research is being extended an order of magnitude in size and scope in attempts to develop a system capable of assessing fate and effects in greater detail and for longer periods of time. The basic system is derived from considerations of the conceptual model of pesticide movement (2) in air, soil, and biota and from the pioneering work of Metcalf's group (1, 3). The Terrestrial Microcosm Chamber (TMC) consists of a box with associated air and water systems and contains an artificial soil and a variety of biota. General design objectives of the TMC have been to provide capability for:

1. Complete mass balance or accountability for an introduced pesticide;
2. Repetitive samples so that the temporal course of disposition could be followed;

Figure 2

NERL Terrestrial Microcosm Chamber (TMC)


(AIR INLET)
"RAIN"
SAMPLING PORTHOLE
(TO AIR MONITORING PACKAGE)
"SPRING"
POTTING MIXTURE
SAND, GRAVEL
(FROM "SPRING" RESERVOIR)
2% GRADE
(TO "GROUND-WATER" RESERVOIR)

BIOTA:

- Annual rye grass
- Alfalfa
- Soybean
- Corn
- Endemic flora (algae, bacteria)
- Endemic micro-fauna (protozoa, etc.)
- Nematodes
- Tenebrio
- Earthworm
- Pillbug
- Field cricket
- Housefly
- Snail
- Anoleus ("Chameleon")
- Button quail
- Gray-tailed vole

3. A situation in which all or a major part of the life cycle of selected organisms could be observed, quantified, and evaluated in the face of chemical insult; and

4. An apparatus and operational system of general availability and applicability to disposition studies, as a screen by industry or the Agency.

The TMC

The TMC (Figure 2) consists of a glass box (1 x 0.75 x 0.61 m deep) with a Plexiglas lid. The glass joints are sealed with silastic aquarium cement covered with aluminum tape. The lid has an access port (20 x 30 cm) in the center covered with a 30 x 40-cm sliding Plexiglas door. Around the upper edge of the port hole and on both the lower and upper surfaces of the door are adhesivebacked Teflon string. The door is held in place by a slide, the lower edge of which is covered with the Teflon tape over a layer of 1/4-inch cork.

The top and bottom exterior edges of the box are bound with nylon-reinforced tape; the exterior bottom 20 cm of the box are also covered with opaque duct tape. The top edge of the box has a 6-mm cork trim covered with an epoxy resin, acting as a seal between the lid and the box. The lid, which is edged and crossed by 1/2 x 1-inch Plexiglas bars to act as reinforcing members, is compressed against the box by 1/4-inch aluminum bars (across the top along the outside edge) connected by threaded rods to the light bank support frame.

The TMC rests on a 1-inch thick polyethylene foam cushion over a plywood frame which is set up to provide a 2 percent grade (2 cm drop per 100 cm) longitudinally. A pair of TMCs are set under a light bank containing 16 96-inch, 100-W blue white fluorescent tubes. A double 100-W pink bulb incandescent fixture is centered over each TMC. The lighting is controlled by a cam timer such that at "dawn" the incandescent bulbs receive variable power (0 to 100 percent) over a 30-minute period followed by sets of four fluorescent tubes being lit at 15- to 20-minute intervals. At "dusk" the procedure is reversed, with the four sets of four tubes being turned off at 15- to 20- minute intervals, followed by dimming of the incandescents. The light/dark period being used is 16 hours on/8 hours off.

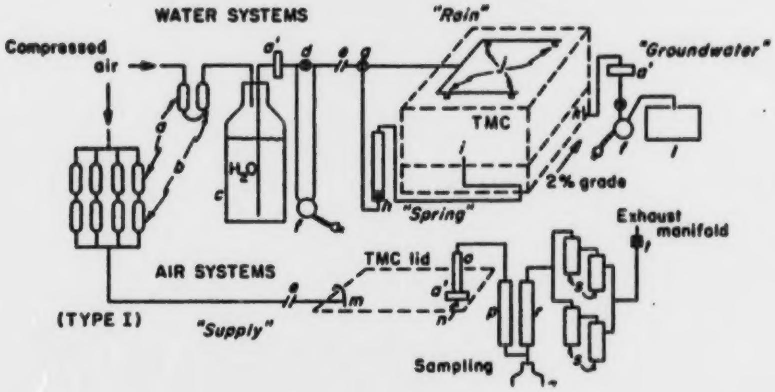
The temperature of the room is regulated by a high capacity air conditioner which provides an ambient temperature of 21-24°C at "night" and 25-30°C during the "day" when the lights are on. The temperature at the soil surface ranges from 24° to 30°C, depending on time of day, degree of foliar coverage, and placement of probe in the TMC, while air temperatures may average about 3° to 5°C higher during the day. The majority of heat input into the TMC is from the light banks located about 30 cm above the lid.

The soil in the chamber has thermistor/psychrometer probes located at various depths. The leads are sealed into the bottom by silastic covered with

Figure 3

Schematic diagram of air and water systems of NERL TMC. a. 5μ filter capsule (a' 47 mm 5μ Metricel filter); b. charcoal filter capsule; c. 25-l. carboy; d. bypass valve; e. equivalent plumbing to each TMC; f. Micropump; g. three-way valve for "rain" and/or "spring" supply; h. needle-valve controlled flowmeter; i. "spring" inlet tube; j. "rain" nozzles; k. "groundwater" outlet tube; l. 10-l. water sample reservoir; m. air supply inlet; n. air sampling system outlet; o. flowmeter; p. 400-mm Graham-type condenser (water-cooled); q. 1-l. Pyrex condensate receiver; r. 400-mm Allihn-type condenser (refrigerated to -15°C); s. gas washing bottles with coarse filter and 300-ml solvent; and t. exhaust vacuum regulating valve.

SCHEMATIC DIAGRAM OF AIR AND WATER
SYSTEMS OF NERL TMC


WATER SYSTEMS
"Rain"
Compressed air
a'
d
e
g
"Groundwater"
a'
a
b
H2O
c
f
TMC
i
j
k
2% grade
h
"Spring"
l
g
f
Exhaust manifold
t
AIR SYSTEMS
TMC lid
o
a'
e
m
n
p
r
s
s
(TYPE I)
"Supply"
Sampling
q

epoxy adhesive. The psychrometer function of all 20 leads ceased after the first few weeks of use, but the thermistor function has remained operable.

The Air System (Figure 3)

Air Supply: The top of the TMC is fitted with a 10-cm nylon-screened (52 x 52 mesh) opening over which a Plexiglas disc has been attached by methylene chloride. The disc has a 7-mm I. D. glass tube cemented into it, which in turn is attached externally to a polypropylene "T"-tube. An excess of membrane- and charcoal-filtered compressed air is delivered at 0.5 psig in the long leg of the "T," replacing air removed from the chamber of the air sampling system (v.i.). The remainder flows out through the other limb of the "T." Should the air supply fail, room air would temporarily replace the filtered air. Should the chamber exhaust fail, the full flow would bypass the chamber. Hence intricate and difficult control of internal TMC air pressure is obviated. A filter or trap may be attached to the external exit of the air system to assess losses in the event of exhaust blockage.

Air Sampling System: The exhaust port in the lid is a 1/2-inch polypropylene Swaglok bulkhead fitting capped on the proximal end with 52 x 52 mesh nylon screening and topped externally with a 47-mm Millipore polypropylene filter holder enclosing a heavy cellulose prefilter and 5µ Metricel filter. Mounted on top of the filter holder is a size 13 Gilmont rotometer, which is used to adjust the air flow rate from the TMC.

A section of polypropylene tubing connects the flowmeter to a standard taper joint into a 400-mm Graham-type water-jacketed condenser. The bottom joint of the condenser is on the side arm of an adaptor mounted on a 1-1 Pyrex bottle and below a refrigerated (-15°C) Allihn-type condenser acting as a freeze-out trap. The refrigeration system is set to automatically defrost for 10-minute intervals every two hours, but that function can be adjusted as needed to prevent blockage. The bottle collects fluids from both condensers. The exhaust exits the freeze-out trap via a standard taper glass joint to a 1/2-inch Pyrex tee attached to nylon Swaglok fittings and passes through a double set of dual gas scrubbers containing 300 ml each of an appropriate trapping solution. The scrubbers are fitted with a 50-mm coarse porous disc located 8 to 10 mm above the bottom of the vessel. Tygon tubing is used to attach the last two scrubbers to an exhaust manifold with a regulating valve. All standard taper joints throughout are sealed with Teflon or Fluochem tapered seals coated at the top with Apiezon lubricant. The freeze-out condenser, scrubber traps, and the refrigeration lines are covered with 1/2-inch black polyethylene foam. The air sampling systems from two TMCs are mounted securely on a single frame which can be easily detached (at the flow meter and exit) to provide better access to the TMC.

The Water System (Figure 3)

The "Rain/Spring" System: Similar to the air system, the water system of the TMC has two parts: one for input or supply and one for output or sampling. Water is added to the TMC as needed from a common supply (reverse-osmosis purified water in a 25-liter carboy) via either a "spring" or by "rain." A three-way valve at each TMC permits both modes of application simultaneously and independently. All components are polypropylene except as noted.

Water is pumped (Micropump) from the reservoir through a 5μ Metricel filter to the valve at the TMC. For the "rain" system the tubing connects to two, parallel-mounted pairs of bulkhead fittings each sealed into the lid 25 cm from the end and 18 cm from the side. The "rain" nozzles are ceramic discs (Bete Fog Nozzle, Inc., Greenfield, Mass.) with 18 0.05-inch tapered and angled (60°) holes. Discs are supported internally by a Viton ring and externally by a Teflon ring seal. Pores in the disc are periodically cleaned by reversing the disc for a short period.

The "spring" system supplies water to a tube extending 1 cm above the surface of the soil. A flowmeter (Gilmont No. 11) is used to regulate the delivery rate of water, which is pumped by air pressure (bypassing the pump at the reservoir). The ascending arm of the "spring" system (located at the high end of the TMC) is encased in a larger (0.5-inch) piece of polypropylene tubing to permit flow directly to the sand-gravel under-stratum. The entry tube is sealed into the bottom of the TMC by epoxy cement over silastic.

The "Groundwater" System: At the low end of each TMC, about 6 cm from each side of one corner, a groundwater sampling tube is sealed through the bottom of the TMC with silastic and epoxy cement. The tube projects inside about 1 cm and is covered by a loose-fitting, 10-cm long section of 1/2-inch O.D. tubing with a 1/4-inch hole drilled in the middle. After exiting the TMC, the tubing ascends to a 47-mm filter holder (containing both a coarse cellulose pre-filter and a 5μ Metricel filter). The line then descends to a shut-off valve and a Micropump, which draws water from the TMC and passes it into a 10-liter sample reservoir.

The Soil

A 5-cm layer (about 40 kg) of washed and autoclaved river gravel passing through a 1/2-inch mesh screen is applied carefully to the bottom of the terrarium. Another 5-cm layer of 8-grit (coarse) washed river sand (about 40 kg) is then overlaid, and both layers are washed with several changes (about 60 liters) of water by pumping off and discarding the water until the effluent is no longer turbid. About 15 liters of distilled water (enough to cover both layers to a depth of about 6 cm) is added carefully with a sprinkling can. The prepared soil or potting mixture is then added in three 40-kg portions along with about 6 liters of distilled water. After each addition of soil or potting mixture and water, a 1/2-inch plywood board (the size of the surface of the TMC and with a small cutout for the "spring" pipe) is laid on the soil and topped with two lead bricks (about 15 kg each). After standing overnight, the bricks and board are removed and the next portion of soil and water is added as before. The soil should be slightly (about 3 cm) below the top edge of the duct tape shielding the sides of the soil from light. A final addition of 2 cm of mixture occurs at planting of the TMC.

Soil thermistor probes are located specifically as to coordinates and depth during the soil addition. Leads must be long enough so that the unburied sensors are out of the way during compaction by the board and so that shielding is provided during operation. Some soil invertebrates are added before the final addition of soil and water.

The soil or potting mixtures utilized thus far have been arbitrary and a matter of experimentation. Washed 20-grit sea sand produced little foliage even when used with a complete hydroponic supplement (1). Invertebrates therein suffered severe abrasion. Standard potting mixtures, such as Jiffy-Mix Plus, have an inordinate amount of organic matter (50 percent peatmoss) and would not be relevant to activity in normal soils. Excellent productivity and compatibility with quail, voles, and various invertebrates were found for this material, however. The currently used mixture is composed (on a weight basis) of 10 percent hydrated Jiffy-Mix Plus, 45 percent 20-grit washed sea sand, and 45 percent Turface (Wyandotte Chemical Co.), a heat-expanded illite clay soil amendment.

The Plants

With approximately 2 cm more soil to be added to the terrarium, the seeds are added by (a) planting according to a specific pattern (coordinates, depth) or (b) scattering a given weight of seeds as evenly as possible over the surface. Species planted according to (a) include corn (*Zea maize*, "Golden Jubilee" or Midget Golden Bantam), peas (*Pisum satrum*), sorghum (*Sorghum vulgaris*), and a variety of kole crops (*Brassicasidae*). Species planted according to (b) have included alfalfa (*Meticago sativa*), Johnsongrass (*Sorghum halpense*), perennial ryegrass (*Lolium perenne*), and annual bluegrass (*Psoa annua*). A wide variety of indigonous algae have been observed, including greens and blue-greens. Their appearance has been discouraged by addition of a fine layer of sand over the top of the final 2 cm of soil added after addition of seeds. However, as the surface is disturbed (e.g., by digging of the vole), the algae become active.

In any given terrarium, a botanical array has been sought which will provide

1. Differential herbicidal or phytotoxic response (broad-leaf vs. grasses);
2. Economic crop (corn, soybeans) vs. weed (ryegrass, Johnsongrass);
3. Sound food basis for vole (ryegrass, alfalfa, corn, soybean), and
4. A conformation supportive of anoles (corn, soybean, Sorghum spp.).

Corn and soybean are two of the crops most intensely impacted by pesticide usage, and a number of the substitute chemicals will find heavy application to these very large acreages. In the Pacific Northwest grass seed crops are of substantial economic importance. Perennial ryegrass has the greatest acreage, but little pesticide usage is required. On the other hand, this plant is one of the most prevalent and economically significant weeds in other cereals in the region. Its growth habit and ease of handling make it an ideal component of the terrarium.

Other grasses are of less importance, either as crops or weeds, but Johnsongrass is a major weed problem in corn and cotton areas of the Midwest and Southeast. It has proven difficult to handle (highly variable germination, propensity for alleopathic effects on other plants) and appears to be less than ideal. Additionally the voles regard it as a less desirable foodstuff.

Alfalfa and other legumes in addition to soybeans do well in supporting a variety of economic insects and are widely grown throughout the country. Their growth habits are excellent for the terraria, since they can form dense plantings of substantial biomass. The vole of choice (v. i.) is native to alfalfa and pastures in the Willamette Valley and prefers these crops to all others offered.

The Animals

Invertebrates

In this first phase of microcosm development the following species have been included for the reasons noted. In general, the criterion of availability from open sources has been followed.

Nematodes: No endemic species of nematodes have been isolated from unsterilized soil mixture. Two easily identified bacteriophagic nematodes have been added in water to the soil system prior to planting: Pristionchus iheriteiri and Cephalobus perseghis. The latter becomes dominant after about 30 days. These species were selected primarily for their ease of culture by the Soil Ecology Unit at NERL. Nematodes occupy a significant point in the food web as consumers of bacteria elicited by plant root exudates, animal wastes, etc., and as food for other soil invertebrates. Phytopathogenic nematodes, while of interest both in theory and practice, could introduce a problem of infestation interrupting the basic energy converting system upon which the principal organism (e.g., vole) is dependent.

Earthworms: Commercially available Lumbricus spp. provide another important link between soil-bound pesticides and other biota. The worms are conveniently and effectively added just prior to the final compaction of the soil. Their activity in redistributing soil residues is very evident at early stages of terrarium growth. Effects of agents on these species are judged significant, since loss of earthworm populations may alter soil character and productivity so markedly. In addition to Lumbricus spp., laboratory-reared Enchaetriad worms are being studied, since the latter have a higher soil temperature tolerance limit.

Snails: The common garden snail, Helix pomata, is readily reared in large numbers. The adults are not preyed upon by other microcosm inhabitants, but the young which emerge from eggs laid deep (15 cm) in the soil are prey for voles and possibly other vertebrates. Being strict herbivores, the snails provide a constant competition for the vole and serve as terminal repository of plant-borne residues. These snails represent the order Mollusca and with slugs constitute a major pest of agricultural and ornamental crops.

Meal Worms: Third and fourth instar larvae of Tenebrio molitar are readily reared or obtained commercially. They are added primarily as a food source for the vertebrates, but their subterranean and scavenging habits also mean that they are exposed to soil-bound pesticides and other residues and hence can effect bio-transfer of these agents or their metabolites. These larvae are added prior to additions of vertebrates and at several times during the life of the terrarium. Excess adults from the laboratory colony have also been added in some tests and fare well.

Crickets: Adult brown house crickets (Achetus domesticus are obtained in large numbers inexpensively, either from commercial producers or by rearing in the laboratory. As omnivorous/herbivorous orthopters, they serve several roles in the microcosm ecosystem, but act primarily as a food source for vertebrates. Their food and activity patterns present opportunity for transfer of residues from soil and plant material to their predators. Reproduction in the soil provides a sensitive index of toxicity, while the young continue to occupy a role in the food web somewhat similar to that of the adults. A substantial portion of the life cycle (10-12 weeks) can be completed in the TMC.

Houseflies: Musca domestica L. are easily reared in quantity and provide an excellent food species for insectivores. The toxicological background knowledge on this species is superior to that of any other included among the TMC biota, since houseflies have been a leading pest and are so easily manipulated. The particular strain employed herein is the maximally susceptible (e.g., lacking genes for dieldrin resistance on Chromosomes II, III, and V), blind (white eye), vestigial winged, mutant. Since these can neither fly nor avoid predators by sight, they are an ideal prey. This strain has been thoroughly investigated in the course of a number of studies on insecticide resistance, degradation, and microsomal induction as detailed in the works of our source, Dr. Terriere's group at Oregon State University (c.f., Schonbrod et al., 1968; Yu and Terriere, 1973).

Pillbugs: Armadillarium and Porcella ssp. gathered in the field and reared in the laboratory are included as representative of the arthropod detritovores. Adults are generally not subject to predation, although cannibalism can be a problem when food is in short supply. Young pillbugs appear to be vulnerable to their own and other species, thus providing a food link between decaying organic matter and higher organisms.

Vertebrates

The greatest concern about dispersive chemical usage relates to effects on higher animals, including man. Selection of appropriate models was based on the following criteria:

1. The principal orders affected should be represented, especially Mammalia and Aves:
2. The species should have an ecological niche not grossly distorted by the physical size and contents of the terrarium;
3. The species should show relatively "normal" physiological processes and behavior under the conditions of the terrarium;
4. They should be generally available or easily reared; and
5. Some toxicological background should be available.

Thus far the species selected qualify according to all criteria but the last.

Gray-Tailed Vole: Microtus canicaudus is a locally native vole (40-60 g), now a resident in the alfalfa and ryegrass fields of the Willamette Valley. Dr. Larry Forslund of Oregon State University has been carrying out extensive reproductive behavior studies on this and other Microtus spp. for the past several years. These voles, which mate well in captivity, have an 18-day gestation period with post-partum estrus; hence gravid females (about 55-60 g) are readily available. Normal litter size is five to seven pups born hairless and blind. Weaning occurs at 15 to 20 days of age.

The vole is sufficiently omnivorous to permit it to consume the entire biotic contents of the terraria (except adult snails and birds). They have been observed eating all plant species tested (except mustard); all invertebrates added except adult snails; quail eggs; and even a two-week old Japanese quail chick (about 30 g) that was found dead in the burrow. The anoles are believed to be eaten by the vole since in only a few instances anoles added to the terraria have been recovered at termination (e.g., at the loss of all surface vegetation). Only two dead anoles have been found in an operational TMC. The intense appetite of this rodent and its ability to adapt well to terrarium life commend it for use in biomagnification studies.

Button quail: Although it was originally intended that the toxicologically well-explored Japanese quail (Coturnix coturnix japonica) should be the avian species in the terrarium, that bird was unsuccessful. On the recommendation of James Keith, Denver Wildlife Center, U. S. Fish and Wildlife Service, the button (or Chinese painted) quail (Excalfactoria chinensis) has been employed in the TMC. Although basically a seed-eater, this species is smaller (40-60 g adult) and more omnivorous than other galliforms which might be used. Males and females are distinctively marked and are handled fairly easily. They are available from most exotic bird distributors and breeders, but have not received systematic attention nor any toxicological study. They lack a cloacal gland and hence should be excellent subjects for artificial insemination (pers. comm. Dr. George Arscott), a matter providing for side experiments using individual males exposed to agents in the TMC.

"Chameleons" (Anoleus sp.): Very little study of the effects or even of residues of pesticides in reptiles has taken place, even though these species can be especially important in a number of ecosystems. Since Anoleus sp. are readily obtained and maintained in captivity and since these are active insectivores, they seem a reasonable component in the terraria. They are arboreal animals, however, and require (or at least prefer) habitats 10 to 30 cm off the ground in the TMC. Heavy stemmed grasses (e.g., corn, sorghum, or Johnsongrass) provide an adequate habitat. They feed only on live insects, adults or larvae, and in turn appear to be preyed upon by the vole.

Results and Discussion

Pesticides have not yet been tested in the TMC system, as efforts to date have concentrated on developing the operational parameters and requisite understanding of the combinations of biotic and abiotic elements. Tests in preparation include studies of sampling procedures and appropriate analyses to ascertain the functionability of the design of both the TMC and experiments. The current test protocol is shown in Table 1 and typical results for such an experiment are illustrated in Figure 4. The average terrarium appears to provide 5-7 kg of plant material in 25 to 30 days. Button quail require supplemental feeding of seeds (10 g of mixed alfalfa and ryegrass) every second or third day, as assessed by body weight changes. Added crickets are consumed quickly (about 10-15 crickets/bird/day). Once the vole is added, however, the competition between the vole and quail requires higher food inputs to prevent starvation of the quail. Then 10 g of seeds and 25 crickets were added daily. Oyster shell (10 g) is added weekly for laying female quail and snails.

The pregnant female vole literally destroys the terrarium. Initial impacts involve clipping of alfalfa and soybean leaves, tunneling and nest building, and in two instances chopping down of the corn plants. The growth of the corn shades the terrarium and substantively alters the soil temperature. Why two voles elected to raze the corn without eating it is unclear, but the degree of unpredictability of such behavior is not tolerable in attempts to generate a consistent microclimate. For 13 other voles, the corn was left until last, and then the leaves were attacked first. Hence, inclusion of corn as a test crop risks consistency of operation which should be taken into account in design of experiments for pesticides not used on corn.

Table 1: Protocol for Typical Experiment

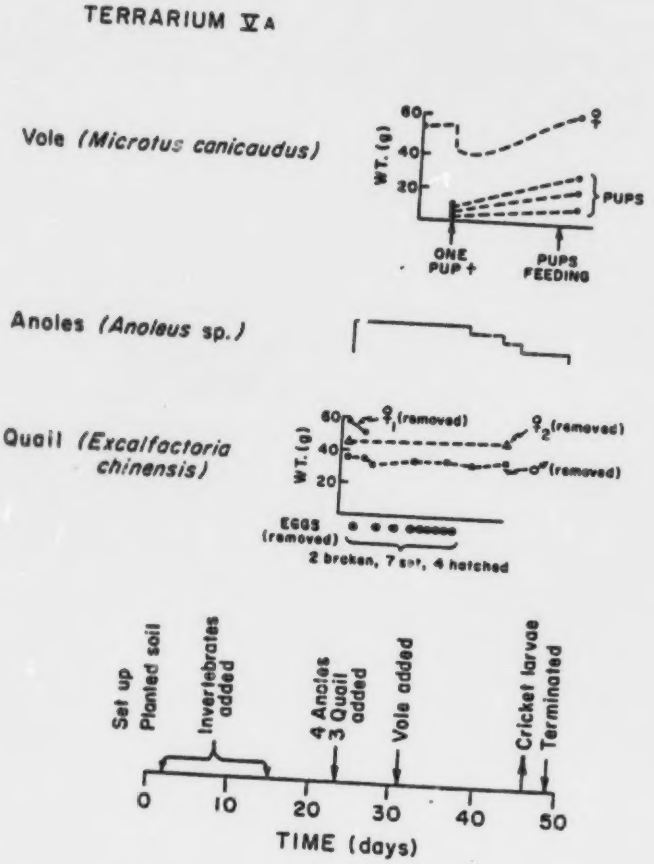
TYPICAL EXPERIMENT

DAY	
-3	Add soil mixture Start light/temperature cycle
0	[Treat with pesticide] Plant seeds Add earthworms, Tenebrio larvae, pillbugs. nematodes Bring water up to 1/3 level by rain
1-3	Bring water up to level by rain
7	Add 150 crickets
14	Add anoles
21	Add male and female quail alfalfa and rye seed crickets Tenebrio larvae houseflies
28	Remove quail Add more seed, crickets Add gravid vole
42-56	Remove vole, terminate terrarium

The voles survive an average of 21.4 days after introduction and produce an average of 4.6 pups per litter brought to weaning. However, some voles have not whelped or the pups have not survived at all. Possibilities for abortion or cannibalism of young must be kept in mind in evaluating effects of any treatment. Where pups have been weaned, the terrarium is characteristically well-tunneled, with a nest about 10 cm in diameter located just above the sand layer deep in the soil toward the upper end of the TMC. Such nests will have several (2-5) entrances and are filled with rye or alfalfa as nesting material. Frequently, but not always, storage areas for food will be found in other portions of the tunnel system.

Figure 4

Results of Typical Experiment



In situations where the vole has not produced or has at least failed to wean pups, the extent of tunneling is much less, the nest has only one entrance, and storage areas are absent. Superficial surface runs or tunnels are always created, particularly near the edges of the TMC. Root feeding has not been evident for this species, except on corn at the later stages of the experiment.

When laying quail and the vole are present, the eggs must be removed shortly after being laid. In one series of tests, the quail produced an average of 0.44 eggs/day in three terraria. About 50 percent hatched when incubated for 16 to 18 days at 38°C. The quail make a rough nest of grass, almost cave-like, which simplifies locating the eggs, apparently laid about the same time each day. When the eggs were not collected in early experiments, they were eaten by the vole. No direct interaction of the quail and vole were observed.

The anoles fared less well, partly because the quail and vole were more aggressive in acquiring the insects (especially crickets and Tenebrio). As the supporting foliage and stems were eaten, the anoles began disappearing. The anoles would display their vermillion throat patch whenever the vole approached them, but no vole has been observed attacking the anoles. The average anole weighs about 2.5 g and would not be able to defend itself except by flight. Even the addition of an artificial "tree" (a cross-tee of 5-mm bamboo set up 10 to 15 cm above the soil surface) did not provide sufficient protection.

The insects survive to varying degrees. The commercially obtained crickets showed 50 percent survival times of about two weeks in the absence of predators. Tenebrio larvae matured to adults, but the latter did not reproduce. The crickets produced large numbers of young which appeared about 15 to 20 days after adults were added. No cricket matured beyond the third instar stage during the course of these experiments (45 to 60 days). Houseflies lasted only one to two days post-emergence due to predation, and the pupae had to be suspended in a container from the lid in order to obtain emergence. Otherwise the vole or quail would have eaten the pupae.

Earthworms did not survive well at the operating temperatures used in these studies, although other tests in the soil mixture at 20 to 22°C gave good (60 percent or better) survival. The vast majority of earthworms recovered have been located at the edge deep in the soil.

Snails survive without incident and almost without exception. Young are not always produced, but egg masses or young snails occurred in aobut 70 percent of the TMCs with 4 or more snails. Snails create a problem in that their feces and slime trails on the box obscure visibility and are difficult to clean off (e.g., for recovery of pesticides). As condensate forms on the top and sides, algae blooms also occur, but these are not suppressed by the snails. The early stages of young snails are eaten by the vole.

Nematodes are under more intensive study because the observed distributions thus far seem to be highly indicative of the extent of the edge effect in the TMC. Concentrations of nematodes in the 5-cm wide zone at the edge, where vole and quail paths provide trampled and decaying vegetation and animal feces, may be up to 100 times greater than in the center top (upper 5-cm) zone. Populations differ by an order of magnitude less at 10 cm depth.

Pillbugs also seem to fare well in the terraria, although survival is limited to about 20 to 30 percent recovered after 45 days. They are usually found on the surface and at the edge, but are active throughout the TMC. Both Tenebrio larvae and pillbugs are found feeding on dead adult crickets and Tenebrio.

The plants also have had varying degrees of success. Moisture must be carefully regulated to low levels to prevent necrotic lesions on the soybean leaves. Soybeans will develop inflorescences but not fully flower under the light conditions; corn will develop spikes, but not tassels. Ryegrass goes to seed after 50 to 60 days. Alfalfa never blooms and growth is retarded relative to field- or greenhouse-grown plants. Bluegrass does moderately well under soybeans, but none of the grasses did well in the presence of Johnsongrass. Three to 30 plants of the latter would sprout from 10 g of seed. The sorghums (S. vulgaris and S. halpense) had the same growth habit as corn and were about as poorly accepted by the vole, i.e., only toward the end of vegetative growth of the TMC. Among the other plants with characteristics suitable to the TMC are included several beans (dwarf lima, cowpea, chickpea, sweetpea, garden pea) which are readily accepted by the vole. The brassicacious plants (several lettuces, cabbages, brussel sprouts, kale, mustard, and spinach) grew slowly and were not well accepted. Carrots and peppers grew very poorly. All plants tested in the currently used potting mixture (Jiffy-Mix/sand/Turface) had heavy root development, in some cases down into the sand/gravel base. Root development was heaviest in sand alone, but foliar parts were minimal. Root development was less in Jiffy-Mix alone, although foliar development was adequate.

Conclusions

Thus far the 15 TMCs have operated in such a way as to provide convincing evidence that the future study of pesticides and other chemicals is feasible in more complex systems than attempted heretofore. Yet to be tested are those portions and operations of the system that involve analysis and treatment with pesticide for determination of the functionability of the TMC in that regard. Still further ahead in testing and design is the mathematical model of the TMC that is being assembled (6). At the end of the first year of effort in the Substitute Chemical Program, the NERL Terrestrial Microcosm project then has developed a conceptual model of pesticide movement, employed that model in design and construction of a terrestrial microcosm test system, and is ready to begin tests with pesticides. The first series of tests will employ ^{14}C- dieldrin and ^{14}C-methyl parathion to investigate aspects of operation. The second series of planned tests will compare the fate of ^{14}C-labeled p-nitrophenol from parathion, methyl parathion, and p-nitrophenol itself. The third series of tests will compare pentachlorophenol, hexachlorobenzene, and pentachloronitrobenzene. This latter group, an analogous series of substituted benzene rings differing only at one group, provide comparisons derived from the consequent physicochemical differences and also are related in pesticidal characteristics. HCB is a major contaminant of PCNB; PCP, a major herbicide in its own right, is also a metabolite of HCB. The fungicide captan (^{14}C-trichloromethyl-labeled) may also be compared in this series since the three benzenoid chemicals all have fungicidal or fungistatic activity.

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QUESTION: You made a statement that you were going to look at the nutrient cycles. What did you have in mind?

DR. GILLETT: Particularly nitrogen, phosphorus, sulfur, and carbon. We have a soil ecology group working on the same processes in litter decomposition in association with coal-fired power plant siting. If they can develop adequate methodologies that are conveniently used, we will probably use them.

QUESTION: Had you considered the effect of these chemicals on this microflora? Were you thinking of that route or are there so many ways to tackle it?

DR. GILLETT: There are so many ways and one of the problems, which I may discuss later about the mathematical simulation, is trying to discriminate between the significant processes by sensitivity analysis of the mathematical model. This requires that several laboratories do a lot more work on ecosystem simulation in the laboratory and in mathematical models.

COMMENT: In answer to your question, we're trying to develop something that will work to look at changes at ATP concentration, changes in direct count to get handles on the bacterial populations and also looking at CO_2 production and oxygen consumption to try to get a handle on the process and process rate.

QUESTION: I can't tell whether you had ultra-violet light there or not.

DR. GILLETT: No, we do not have and that is a problem in terms of photolysis.

QUESTION: What is the effect on your controls of possible starvation of your organisms during the tests? For example, a quail might run out of food or the vole might eat most of the food toward the end. Mice sometimes will have reproductive effects if they are starved.

DR. GILLETT: Regarding our voles, we could have a real problem. For instance, once the last bit of food disappears, the pups are extremely vulnerable. She has a litter of six or seven and they are about five to seven grams. The demands on the mother and the demands on the system are extraordinarily high.

The pups can die quickly because they do not have enough energy input. We have to find means of identifying where she is and what her condition might be. We are considering a thermistor telemetric device which will measure her body temperature, because if that starts dropping, then her energy consumption is abnormal. The device will tell us not only where she is but also something about her condition. That is getting very sophisticated in terms of what we need to know about disposition and may not be worthwhile in terms of the overall cost of the experiment.

You have to understand the processes within this chamber just as you must understand the processes in the environment. You can intuitively and deductively reach some understanding of what is happening. This system does not provide answers; in the absolute sense it provides guidance to where to ask the best questions and what to look for. Whether biomagnification or a multiplicity of metabolic pathways are present, this will reveal it. It will not tell you the routes of metabolism in cows. It will not tell you what will happen in mothers' milk, but it will give you a profile around which you can build a good research program.

PROGRESS AND STATUS REPORT ON TERRESTRIAL MICROCOSM DEVELOPMENT

Dr. James R. Sanborn*

NOT AVAILABLE

FOR

PUBLICATION

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*Illinois Natural History Survey, Urbana, Illinois

THE EFFECTS OF MIREX ON THE PREDATOR-PREY INTERACTIONS IN AN EXPERIMENTAL ESTUARINE ECOSYSTEM

Dr. W. Peter Schoor*

This report has not been published and its contents must therefore be considered provisional.

The reason for using mirex is to establish an exposed control type system. That is the point at which we now are.

A simple model ecosystem was developed to study the fate and effects of pollutants in the estuary. The approach used is intermediate in complexity and represents a refinement beyond single-species bioassays in that a food chain permits the study of the ability of an animal to escape predation after exposure to nonlethal concentrations of such compounds. We have thus made use of one of the most fundamental, yet most sensitive, biological indicators of behavior.

This criterion of effect has been largely disregarded in recent years, especially since the advent of molecular characterization. The present state of fundamental biochemical research, however, permits the use of enzymatic reactions as unequivocally sensitive tools only in rare cases, especially outside of mammalian pharmacology and toxicology.

Metcalf et al. (1971) described an experimental fresh water ecosystem which has produced valuable data on the biodegradation and bioconcentration of many compounds. Our experimental ecosystem is similar to theirs and will be used as a screening device for toxic materials to identify the most toxic and/or most highly concentrated compounds that would be potentially hazardous to the estuarine environment. This ecosystem can serve until a scientifically more rigorous and elaborate system has been developed to assess more accurately detrimental effects of pollutants on the estuary. We are presently engaged in this task, but find that nature steadfastly refuses to be simulated on a short-term basis.

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*Gulf Breeze Environmental Research Laboratory, Estuarine Systems Branch

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Experimental

The components of our ecosystem represent a simple food chain that includes a primary producer (turtle grass - Thalassia testudinum), a primary consumer (grass shrimp - Palaemonetes vulgaris), and a secondary consumer (pinfish - Lagodon rhomboides). This choice represents an important part of the food web found in turtle grass communities (Hansen, 1969; Adams and Angelovic, 1970) which, ranging in depth from 0.5 to 4.5 m, cover much of the bottoms in estuaries of the northern Gulf of Mexico (Humm, 1956). It also assures a year-round, continuous and reliable source of organisms which adjust readily to laboratory conditions.

Mirex was chosen as toxicant because it is a chemically fairly stable compound not readily hydrolyzed, oxidized, reduced, or otherwise chemically altered. This stability permits long-term exposures with reasonable assurance that the parent compound will be the one actually investigated. Few other compounds interfere directly with its quantitative analysis by gas chromatography. Moreover, mirex can also be introduced into the water without the aid of a solubilizer, since it is used in bait form adsorbed to corn cob grit. Any induced leaching thus provides amounts of mirex in the water as they would occur in the environment.

The physical size of the system was chosen to allow water samples of 1 to 2 liters to be taken without undue disturbance of the "steady-state." It is also the minimum size in which to keep a community consisting of the following components:

- 160 liters of artificial seawater (distilled water and marine mix) at a salinity of 20 parts per thousand.
- 4 cm of sand. Stock of sand had been dredged from Santa Rosa Sound, Florida, and consisted of 25 percent coarse particles (#35 standard sieve) and 75 percent medium particles (#120 sieve).
- 75 turtle grass plants, Thalassia testudinum, (175-210 g total) planted over 1500 cm^2 of the bottom.
- 75 adult grass shrimp, Palaemonetes vulgaris, (30-35 mm rostrum-telson length, 0.20-0.25 g each).

- 2 juvenile pinfish, Lagodon rhomboides, (90-95 mm total length, 9-12 g each).

The tanks were covered and water temperatures maintained at 20±1°C. Fluorescent lights were used to provide 12-hour periods of light and dark. The dissolved oxygen content was kept relatively high by artificial aeration. The temperature, pH, dissolved oxygen, and turbidity were measured at regular intervals.

After equilibration of the system for 4-6 days, mirex was introduced into the water by means of an air-lift column, in which a continuous flow of air causes water to percolate through a column containing mirex in bait form (Borthwick, 1975). The amount of mirex in the columns represented five times field-rate application and was chosen because earlier studies (Tagatz *et al.*, 1975; Borthwick, 1975) showed that concentrations thus produced in the water did not cause significant mortalities in grass shrimp exposed for a period of 3 weeks.

Water, sand, turtle grass, and shrimp were sampled at intervals (Table 1), and mirex residues were determined using a Hewlett-Packard Model 5700 gas chromatograph with a ^{63}Ni electron-capture detector. An OV 101 column (2 percent OV 101 on gas chrom Q, 100-120 mesh) was operated at 195°C with the detector at 300°C and the argon/methane (10:1) carrier gas at a flowrate of 60 ml/min.

Table 1

Exposure (Days)	Amount Mirex (μg)[1] Shrimp[2]	Amount Mirex (μg)[1] Water
1	0.4	15
4	1.1	16
7	2.0	12
10	2.6	9.2
13	2.5	5.0

---

[1] Simple average of two replicate tanks.

[2] Total residue in component remaining at corresponding length of exposure.

Results

After an exposure period to mirex of 16 days, chi-square analysis of the surviving shrimp showed nonsignificant mortalities in all tanks (Table 5). Predators (pinfish) were added at that point and mortalities of shrimp observed at intervals of 1, 2 and 3 days. The results are depicted in Table 5.

The water quality data indicate that our experimental ecosystem was not significantly stressed, if at all. The pH values are normal for those of seawater (7 5-8.4) and the dissolved oxygen was close to saturation. The water was not turbid. Appearance of new blades of turtle grass indicated growth under test conditions in both control and exposed tanks.

The amount of mirex in the water was deliberately chosen to reflect nonlethal levels for the time of exposure, which was substantiated by chi-square analysis. This permitted predation on grass shrimp which were not dying, but obviously affected by mirex to such an extent as not to be able to escape predation to the same degree as control animals. Chi-square analysis showed significance at the 5 percent and 1 percent levels.

Table 2

Exposure (Days)	Amount Mirex (μg)[1] Shrimp[2]	Thalassia[2]	Sand	Total	Water
1	1.0	1.4	8.4	11	20
4	2.0	2.4	18	22	9.1
7	2.6	2.2	14	19	4.9
10	1.6	2.3	31	35	4.7
13	2.1	1.7	43	47	2.9

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[1] Simple average of two replicate tanks.

[2] Total residue in component remaining at corresponding length of exposure.

Table 3: Averages ($\mu g/kg$) of Mirex

Exposure (Days)	Hepato-pancreas	Exo-skeleton	Muscle	Head	Thalassia	Water	Sand	WBR
	Plants No Plants	Plants No Plants	Plants No Plants	Plants No Plants	Detritus Leaves	Plants No Plants	Plants	Plants No Plants
1	280 50	120 40	40 20	110 80	17 8	0.12 0.088	0.30	100 40
4	370 240	200 100	90 30	200 100	28 18	0.057 0.10	0.65	160 100
7	360 580	320 300	180 120	280 270	27 17	0.031 0.073	0.50	260 230
10	500 748	180 230	60 120	130 260	31 13	0.030 0.058	1.10	140 220
13	650 880	150 210	80 140	120 150	29 15	0.018 0.031	1.50	130 200

Table 4: Adjusted (Water) Averages (μg/kg) of Mirex

Exposure (Days)	Hepato-pancreas	Exo-skeleton	Muscle	Head	Thalassia	Water	Sand	WBR
	Plants No Plants	Plants No Plants	Plants No Plants	Plants No Plants	Detritus Leaves	Plants No Plants*	Plants	Plants No Plants
1	280 70	120 60	40 30	110 110	17 8	0.12 0.12	0.30	100 50
4	370 140	200 60	90 20	200 60	28 18	0.057 0.057	0.65	160 60
7	360 250	320 130	180 50	280 120	27 17	0.031 0.031	0.50	260 100
10	500 380	180 120	60 60	130 140	31 13	0.030 0.030	1.10	140 110
13	650 510	150 120	80 80	120 90	29 15	0.018 0.018	1.50	130 120

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*Adjusted

Table 5

Tanks Control/ Treated	Mirex (µg/l)	% Survival (Numbers)		Chi-Square[2]	Predation (Days)	% Survival (Numbers)		Chi-Square[2]
		Control	Exposed			Control	Exposed	
1/1	0.051 (0.13-0.019)	82 (52 of 63)	68 (43 of 63)	N.S.	1	44 (23 of 52)	23 (10 of 43)	4.50[a]
1/1	0.051 (0.11-0.017)	78 (49 of 63)	81 (51 of 63)	N.S.	2	16 (8 of 49)	0 (0 of 51)	9.14[b]
3/3	0.025 (0.05-0.015)	94 (177 of 189)	88 (167 of 189)	N.S.	3	24 (42 of 177)	4 (5 of 115)[3]	19.4[c]

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[1] Average concentration of mirex throughout exposure period.

[2] N.S. = nonsignificant; a = significant at 5% level (x^2, 1 d.f. = 3.84); b = significant at 1% level (x^2, 1 d.f. = 6.63).

[3] Omitted 1 tank because one pinfish died.

Discussion

We find, thus, that while the criterion of death may be useful in single-species bioassay, in an ecosystem bioassay an effect on the predator-prey interactions may have far greater implications regarding the total welfare of the system. In addition, concentrations at which this behavioral effect occurs are quite low and come closer to the environmental levels.

EFFECTS OF MIREX ON THE BURROWING ACTIVITY OF THE LUGWORM (ARENICOLA CRISTATA)

Dr. W. Peter Schoor*

This report has not been published and its contents must, therefore, be considered provisional.

The reason for using mirex is to establish an exposed control type system. That is the point at which we are now.

It has been common practice in the past to classify the substratum of the aquatic environment as a "sink" for pesticides and heavy metals. Yet benthic organisms abound and it is recognized that many of these organisms are instrumental in the physical turnover of this substratum. It was thusly of interest to investigate:

1. Whether or not the substratum represents a "sink" to benthic organisms,
2. What behavioral changes may occur in these organisms in a pesticide-laden substrate, and
3. If pesticides can be channeled back into the pelagic system.

We chose the lugworm (Arenicola cristata) for our studies because of its relative abundance in the shallow estuaries of Northwest Florida. According to D'Asaro (1974), the following are characteristics of the lugworm:

1. The estimated density is six to ten adults per square meter;
2. Except for ease of damage to external gills, they are a hardy species;
3. They adjust well to laboratory conditions, including reproduction; and
4. They are easily maintained on a diet of dried, ground turtle grass (Thalassia testudinum).

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*Gulf Breeze Environmental Research Laboratory, Estuarine Systems Branch

Methods

Two 180-liter covered glass tanks (120 x 30 x 50 cm) were filled to a height of 30 cm with alternating layers of sand (75 percent coarse particles - #35 standard sieve; 75 percent medium particles - #120 standard sieve) and organic silt (from pristine *Spartina* marsh). A photo-period of 10 hours light and 14 hours dark was established using four 40 watt fluorescent bulbs mounted 30 cm above each tank. Filtered seawater, average salinity 26 ppt, was added to fill the tanks. The temperature of the water was maintained at 20 ± 2° C. Algae of the genus *Ectocarpus* were blended and introduced into the tanks to form a growing mat on the substratum. Four adult worms were then introduced in each aquarium and allowed to adjust for 3 days, after which time one tank was exposed to mirex by means of an air-lift column (Borthwick, 1974). One-liter water samples (replaced water) were taken 5 days per week and analyzed for mirex by gas chromatography.

In order to make observations of the surface activity of the worms, vertical lines, 10 cm apart, were drawn on the outside of the tanks ending precisely at the surface of the substratum. The tanks were in this fashion divided into 12 equal transects perpendicular to the tanks' longitudinal axes. Daily sightings were made along each transect, and the following surface features recorded:

1. Active head shafts (feeding funnels),
2. Inactive head shafts,
3. Tail shafts (respiratory holes), and
4. Egg masses.

Each feature was recorded together with the time of appearance and, given a significance of one, recorded *in toto*. The exposure to mirex was discontinued at day 30, and fresh algae were reintroduced in both tanks at days 37 and 55. The experiment was terminated at day 75.

Results and Discussion

Table 1 shows the concentrations of mirex in the water of the exposed tank. The variation seen is most likely due to the adsorption of mirex to particulate

Table 1: Mirex Concentrations in Water

Days Elapsed	Mirex Conc. µg/l
2	0.062
5	0.039
7	0.024
8	0.026
9	0.025
12	0.013
13	0.011
14	0.032
15	0.022
16	0.020
19	0.022
20	0.024
21	0.036
22	0.048
23	0.025
26	0.013
28	0.016

matter (some ultracentrifugal experiments suggest as much as 80 percent), possibly the algae. Our limit of detection for 1 liter of water samples was 0.003 µg/l.

Table 2 represents the total of the surface features appearing per day throughout the duration of the exposure to mirex. As pointed out previously, each feature was assigned a relative significance of one in order to quantitate a behavioral effect. This "relative" significance most likely can be better defined after more

Table 2

LENGTH OF EXPOSURE (DAYS)	EXPOSED							CONTROL						
	●	■	◆	△	DAILY TOTAL	5-DAY TOTAL	DAILY AVG.	●	■	◆	▲	DAILY TOTAL	5-DAY TOTAL	DAILY AVG.
WORMS ADDED						28	5.6						30	6.0
2	2	3			5			1	3			4		
MIREX ADDED	4	2			6			4	12			16		
4	3	1		3	7			4	1			5		
5	3	2	5		10			4	[illegible]			5		
6						26	5.2						35	7.0
7														
8	7	7	2		16			10	4	11		25		
9	1				1			1	1	1		3		
10	7		2		9			2	4	1		7		
11	3	2	2		7	13	2.6	3	2			5	21	4.2
12	2				2			5				5		
13														
14														
15	4				4			6	4	1		11		
16	6	2	1		9	18	3.6	3	2			5	19	3.8
17			2		2			5	4			9		
18	5	2			7			4		1		5		
19														
20														
21	3	2			5	14	2.8	5	4			9	30	6.0
22		3			3			2	1	1	1	5		
23			1	2	3			4	1	1		6		
24	1				1			2	2			4		
25	1	1			2			3	2	1		6		
26						3	.6						18	3.6
27														
28	1	1			2			3	4			7		
29	1				1			2	1	2		5		
30					0			2	3	1		6		

experimentation; however, used as defined here, statistically significant behavioral changes occur after day 23. The exposure to mirex was terminated at day 30, and both tanks observed for another 45 days. On day 37 more algae were introduced

Conclusion

It has thus been shown that:

1. The burrowing activity of Arenicola can affect the distribution of mirex in the substrate;

2. Low concentrations of mirex in water can affect burrowing behavior as measured by surface activity; and

3. The free-swimming juvenile stage can act as a source of mirex to any predator.

DR. GLASS: If I could just address a couple of questions in general that were raised earlier, I think most of the answers to your questions about stability and the reasons for using microcosms are contained in the theory and lore that go along with microcosm development, which started many decades ago, and partly some of the problems we've had experimentally are the result of working with microcosms. We don't plan them to be the end all of experimental apparatuses but they are inexpensive and fairly rapid and useful in many respects.

PROGRESS AND STATUS REPORT ON FRESH WATER ECOSYSTEM METHODOLOGY DEVELOPMENT

Dr. Allan R. Isensee[*]

The contract[**] that our laboratory has with the EPA on the Substitute Chemical Program was finalized in late May 1975. Progress thus far has involved methodology development and the search for a biologist to work on the project. One of the primary contract objectives is the development of a system that incorporates the useful aspects of both the static and flowing ecosystems. The static, as presently employed in this laboratory, subjects a pesticide to some typical environmental conditions in order to determine their effect on the behavior and accumulation of the compound by ecosystem components. The flowing systems have usually been designed to study the effect of the compound on a component of the environment. If some of the major elements of these systems can be "married," the result should be a more comprehensive model ecosystem. This report will describe the ecosystems that we have used and how these systems will be used and modified in relation to goals of the Substitute Chemical Program.

Ecosystem Design

The model ecosystems used in this laboratory (1,2) are entirely aquatic and contain organisms representing parts of two food chains (algae to snails, and daphnids to mosquito fish or fingerling bluegill). The ^{14}C-labeled pesticide is adsorbed to soil (100 grams), introduced into the system, and flooded with 4 liters of water. This mode of entry simulates the major route of unintentional entry of pesticides into the aquatic environment, namely, erosion of soil from agricultural land that has been treated with pesticides. The surface soil, which usually contains the highest concentration of pesticide, is also the most susceptible to erosion. This rationale of pesticide introduction is also useful in determining what soil concentration to use. Based on the behavior of a pesticide in soil, e.g., mobility, adsorption, and rate

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[*] Beltsville Agricultural Research Center, Agricultural Environmental Quality Institute, USDA

[**] Contract No. IA6-D5-F811, Fresh-Water Micro Ecosystem Development and Testing of Substitute Chemicals

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of degradation, and its method of application, an estimate of the concentration in the surface few millimeters or centimeters can be made. We normally use this estimated field concentration, a 10 x rate, and a 10^{-1} x rate. The actual water concentration of pesticide depends not only on the amount of pesticides added, but also on the rate and extent of desorption from soil.

The tanks are placed in a shallow water bath (22+2°C) in the greenhouse and receive only natural light. One day after flooding, daphnids, snails, and algae are added. Water samples are taken three times a week and analyzed by standard liquid scintillation methods. The system is allowed to operate for 30 days. At 30 days samples of daphnids are taken for analysis and two mosquito fish or fingerling bluegill are added. The system is terminated 3 days later when the fish have consumed all daphnids and all organisms are harvested. In some studies, two fingerling channel catfish are added and exposed for an additional 6 days after the other organisms are harvested.

The fish and snails are homogenized in methanol and 1-ml samples are analyzed by standard liquid scintillation methods. If a high concentration of pesticide is indicated, the extract is further analyzed by thin-layer chromatography to determine if and how much metabolism has occurred. The algae and daphnids are assayed for total radioactivity by determining $^{14}CO_2$ from standard combustion and scintillation methods. The results are expressed as ppm (of the parent compound based on ^{14}C content) in the fresh tissue and the bioaccumulation ratio (conc. in tissue/conc. in water) calculated.

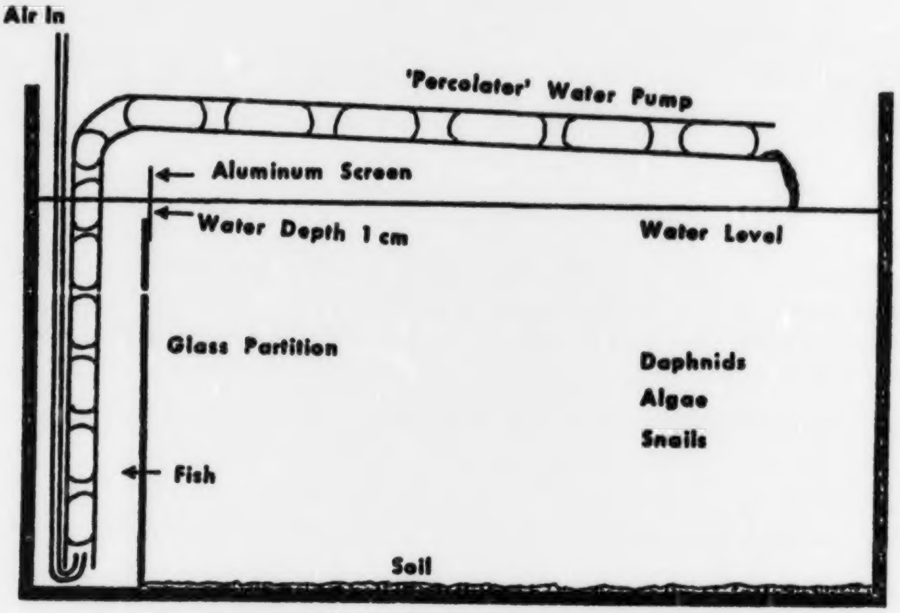
Larger systems have also been used (4), usually containing 10 kg of soil, 80 liters of water, and fingerling channel catfish and crayfish in addition to the organisms used in the smaller systems. This larger system is useful in obtaining more biomass (for metabolism work) and can support larger organisms. The exposure time and methods of analysis are similar to those used in the smaller systems.

Two modified systems are presently under development. One is a recirculating static model ecosystem (Figure 1). Its major advantage over the above systems is that fish are exposed for the entire 30-day period along with the daphnids and other organisms. A 3-day exposure may not be sufficient for equilibrium to occur.

In addition, fish placed in a system after a 30-day period may be exposed only to pesticide metabolites; thus, uptake of compounds by the fish after 30 days may differ qualitatively and quantitatively from that occurring during a continuous 30-day exposure. Some daphnids pass through the screen to provide food for the fish and thereby complete the daphnids to fish food chain; enough daphnids remain to maintain a reproducing population. The number of daphnids that move through the screen is inadequate to provide their entire food supply, thus requiring supplemental feeding. The pump is designed to maintain the same concentration of pesticide on both sides of the glass partition and to transport fecal matter to the larger chamber where daphnids may use it as food. This system has a total water volume of 16 liters but could easily be designed for any convenient size.

Figure 1

RECIRCULATING STATIC MODEL ECOSYSTEM


Air In
'Percolator' Water Pump
Aluminum Screen
Water Depth 1 cm
Water Level
Glass Partition
Daphnids
Algae
Snails
Fish
Soil

Initial testing (minus pesticides) has shown it to be satisfactory, except that the daphnid population was unstable. One pesticide, hexachlorobenzene, has been investigated in both the static and recirculating systems (Table 1). Statistically identical results were not obtained, but treatment conditions (soil aged 1 year vs. freshly treated) were different and may be partly responsible for the different results. However, all results were well within an order of magnitude, which is often cited as the normal variation of model ecosystem-derived data.

Table 1: Comparison of the Static and Recirculating Static Model Ecosystem for the Accumulation of ^{14}C-HCB by Several Organisms

Soil Treatment	H_2O Conc.	Concentration			
		Algae	Snails	Daphnids	Fish
ppm	ppb	ppm	ppm	ppm	ppm
Static					
10[a]	1.72	1.561±0.190	2.805±0.252	1.612±0.359	3.509±0.550
Recirculating Static					
9.2	7.9	4.51±0.46	0.58±0.17	0.95±0.10	3.20±0.69

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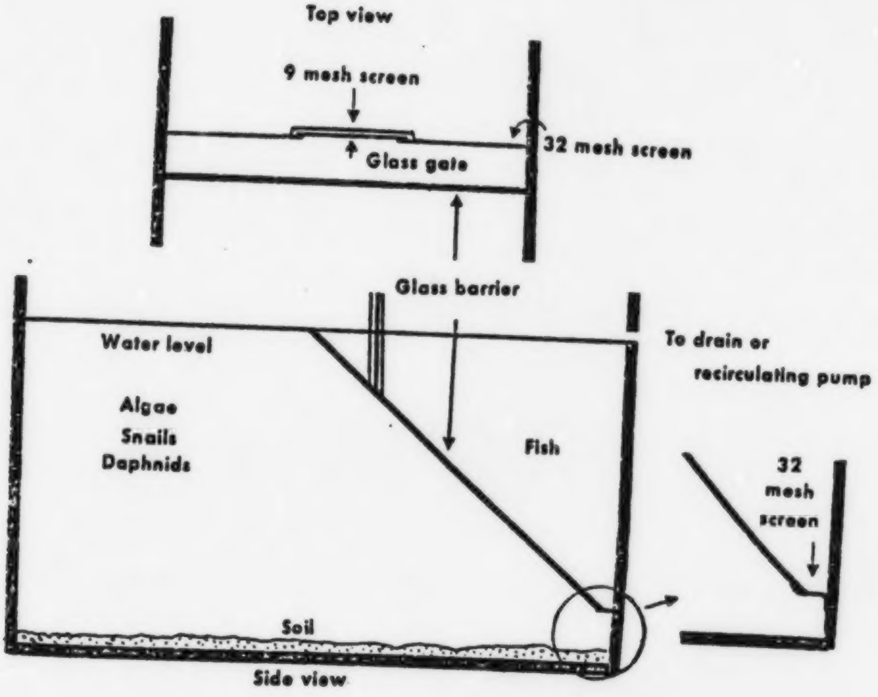
[a] Concentration of ^{14}C-HCB in 100 grams soil treated 2 days before use (static system), and concentration of ^{14}C-HCB in 500 grams soil aged 1 year (recirculating static system). The static and recirculating static systems contain 4 and 16 liters of water, respectively.

[b] Average of three replicates plus or minus the standard error of the mean.

A further modification, shown in Figure 2, is designed to maintain a stable daphnid population and be used as either a static or a flowing ecosystem. The system as shown has a total volume of 60 liters. The major difference between the two systems is the screen and gate combination and the slanting glass barrier. The 32-mesh screen restricts most daphnids while the 9-mesh screen allows all daphnids to pass. The gate can be opened and closed for such time as to release enough daphnids to contribute to the food chain, yet not deplete their population. Fecal matter collects on the lower screen where it disintegrates, passes through the screen, and provides food for daphnids. The system shown in Figure 2 is connected to a serial diluter and has been operated for about 30 days with favorable results. All organisms, including daphnids, thrived in the system.

Figure 2


Top view
9 mesh screen
Glass gate
32 mesh screen
Glass barrier
Water level
To drain or
recirculating pump
Algae
Snails
Daphnids
Fish
32
mesh
screen
Soil
Side view

The serial dilution apparatus that has been constructed for this project is similar to that described by Mount and Brungs (3) and is designed to deliver the test chemical at concentrations of 0.5, 0.1, 0.05, and 0.01 of the highest concentration plus an untreated control.

The next phase of the study will be to start the actual testing and comparing of these static and flowing systems using simazine and trifluralin. These initial studies will be designed to determine how smoothly the systems operate and whether there are any major differences in the behavior and accumulation of chemicals between organisms exposed in the static and flowing systems.

References

1. Isensee, A. R. and Jones, G. E.: Distribution of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) in aquatic model ecosystem. *Environ. Sci. Tech.* 9:668-672 (1975)
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3. Mount, D. I. and Brungs, W. A.: A simplified dosing apparatus for fish toxicology studies. *Water Res.* 1:21-29 (1967)
4. Schuth, C. K., Isensee, A. R., Woolson, E. A., and Kearney, P. C.: Distribution of ^{14}C and arsenic derived from (^{14}C) cacodylic acid in an aquatic ecosystem. *J. Agr. Food Chem.* 22:999-1003 (1974)

DR. MACEK: Are you going to make any effort to try to discriminate between the residue that you detect in an atropic level that comes directly from uptake of water as opposed to that portion of it that may be a function of transfer by the food chain? And in the experimental design, are you going to discriminate between those two sources?

DR. ISENSEE: No, I don't intend to with this system. The concentrations we will work with in the flowing system will probably be based on the concentrations we get from soil in the recirculating static. The flowing system will be tested with and without soil, allowing the soil to have its own interaction, act perhaps as a sink, perhaps to stimulate microbial breakdown. At least four organisms, representing two trophic levels, will be used to measure accumulation and the results compared to the data obtained from the recirculating systems.

MR. JOHNSON: What's the function of all of the rather large number of trophic levels?

DR. ISENSEE: These are the organisms we have worked with in the past and we feel it is better to obtain accumulation data on several organisms rather than one.

QUESTION: What do you do if you use a pesticide more toxic to daphnia than it is to fish?

DR. ISENSEE: In those cases you will operate without some component of the system. However, the whole idea is to work with concentrations which will be realistic in terms of toxicities. For example, methyl parathion has an LD_{50} for daphnids of 4.8 parts per billion, and about 8 parts per million for fish. Therefore, in order to have daphnids surviving, the operating concentration should be in the 1 or 2 parts per billion range.

QUESTION: When you're separating your fish and other components, how do you handle the nutrition of the fish during that period?

DR. ISENSEE: We'll have to go to supplemental feeding because the system obviously does not generate enough daphnia to feed the fish.

QUESTION: What type of supplemental feeding?

DR. ISENSEE: Commercial fish chow.

QUESTION: How stable are the populations of organisms?

DR. ISENSEE: We've run the flowing system for 30 days and gotten blooms of daphnids. Apparently there is enough fecal material from fish plus the effect of soil to get a good daphnid population.

QUESTION: Are the experiments carried out in the greenhouse?

DR. ISENSEE: Yes. Due to space limitations, all experiments are performed in the greenhouse. This results in some algae problems, but has the advantage of allowing photodegradation to occur. Some compounds have been photodegraded in our systems.

MR. JOHNSON: If your daphnids are able to reproduce fairly well and develop a large population, that could result in a really interesting shunt. Then for compounds that are not very stable, the daphnids could be taking compounds out of the water and doing other things to them. I was just curious if you could reproduce that aspect of it.

DR. ISENSEE: With a flowing system you never have to worry about concentrations because you have the toxins coming in all the time. To a certain extent the soil also works well, acting as a reservoir from which the compounds desorb and supply the toxin to the water.

QUESTION: In 30 to 60 days, are unstable populations a problem?

DR. ISENSEE: We have normally had stable populations in the small four-liter tanks.

REVIEW OF RESEARCH PLAN FOR WHOLE SYSTEMS MODEL--DESIGN, VALIDATION, AND INTEGRATION BETWEEN TERRESTRIAL AND AQUATIC ENVIRONMENT

Dr. Erik D. Goodman*

Society is becoming acutely aware of the necessity to develop a means of anticipating environmental problems. Post facto environmental management risks an increasing array of undesirable and perhaps irreversible consequences. The impact review process initiated by the National Environmental Protection Act clearly emphasizes anticipatory decision-making.

Despite the clear need for such an anticipatory decision paradigm based on sound guidelines, in most cases the scientific structures required to predict responses of biological systems have not yet been developed. Predicting these responses requires understanding of the basic mechanisms of component processes and how these are constrained by environmental conditions. Most environmental impact assessments to date have relied on correlation or black box simulation models that fail to reveal the structure-function relationships. The mechanism-oriented approach to be utilized in this research will attempt to represent each process as realistically as is practical, given constraints on data and complexity, and without resorting to a single mathematical form, e.g., linear relationship, to represent all processes.

Over the short term, the research will build upon existing developments in lake modeling at Michigan State University (MSU); current lake models will be revised and expanded to include the effects of pesticides introduced directly into the lake for aquatic pest control and pesticides applied terrestrially that reach lakes in sufficient quantities to represent potential problems. Over the longer term, the research focus will be extended to include the terrestrial impacts of selected pesticides and the dynamics of the pesticides' transport and breakdown. Due to the relatively limited state of knowledge of quantitative ecosystem dynamics in a terrestrial setting, it will take considerably longer to develop a process-oriented predictive model to assess this type of pesticide impact; however, even early explorations should point out critical areas for investigation in the field and laboratory.

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*Department of Electrical Engineering and Systems Science, Michigan State University

A thorough literature search is under way as the first step of the program, and the relevant data obtained will be employed to develop preliminary computer models. The relative importance of the various parameters will be assessed, and the sensitivity of the models to parameter magnitudes will be tested by mathematical modeling and computer-simulation techniques used in the current eutrophication studies at MSU. Computer models will provide the framework for organizing and integrating the information gathered, insuring that missing or incompatible data are recognized as requiring further study.

The literature review will include as "principal" pesticides, malathion, parathion, fenthion, azinphosmethyl, simazine, and atrazine, and as "subsidiary" pesticides, methyl parathion, chlorpyrifos, diazinon, trifluralin, PCNB, and captan. Effects of all six "principal" pesticides and of some or all of the six "subsidiary" pesticides will be modeled by focusing on:

1. The amounts of the selected pesticides which enter the system;
2. The chemistry of the pesticides in their environment;
3. The distribution of the pesticides within the system, as mediated by physicochemical processes;
4. The direct effects of the selected pesticides on specific target and nontarget organisms in the system;
5. The interrelationships among biotic components of the system (for example, among phytoplankton, zooplankton, bacteria, aquatic insects, and fish);
6. The indirect pesticide effects (e.g., food-chain-mediated) on the structure and function of the community; and
7. The degree to which pesticide impacts are modified by the turnover rates of the biotic components (e.g., in a eutrophic vs. oligotrophic lake or fertilized vs. unfertilized field) or by other stresses on components of the ecosystem.

In all cases, causative factors will be sought and characterized. Special attention will be paid to both direct and indirect responses of the various elements of the food web to the pesticide.

The approach to be followed is indicated by the structure being developed for physical and biological processes in the lake ecosystem (Figure 1). Variables not homogeneously distributed in the lake are maintained separately for each structural element, i.e., ring or layer. For example, temperature, light, pesticide residue level, population densities of several fish, algae, zooplankton, insects, and macrophytes, and several chemical concentrations in each element are calculated at each time step. Movement between adjacent elements occurs by passive mixing as well as by locomotion where appropriate. Spatial heterogeneity must be explicitly represented, since many feedbacks between biological and physical processes are often expressed via changes in the spatial distribution of materials and energy. The level of structural detail (i.e., number of layers), however, can be chosen to fit the particular problem under study.

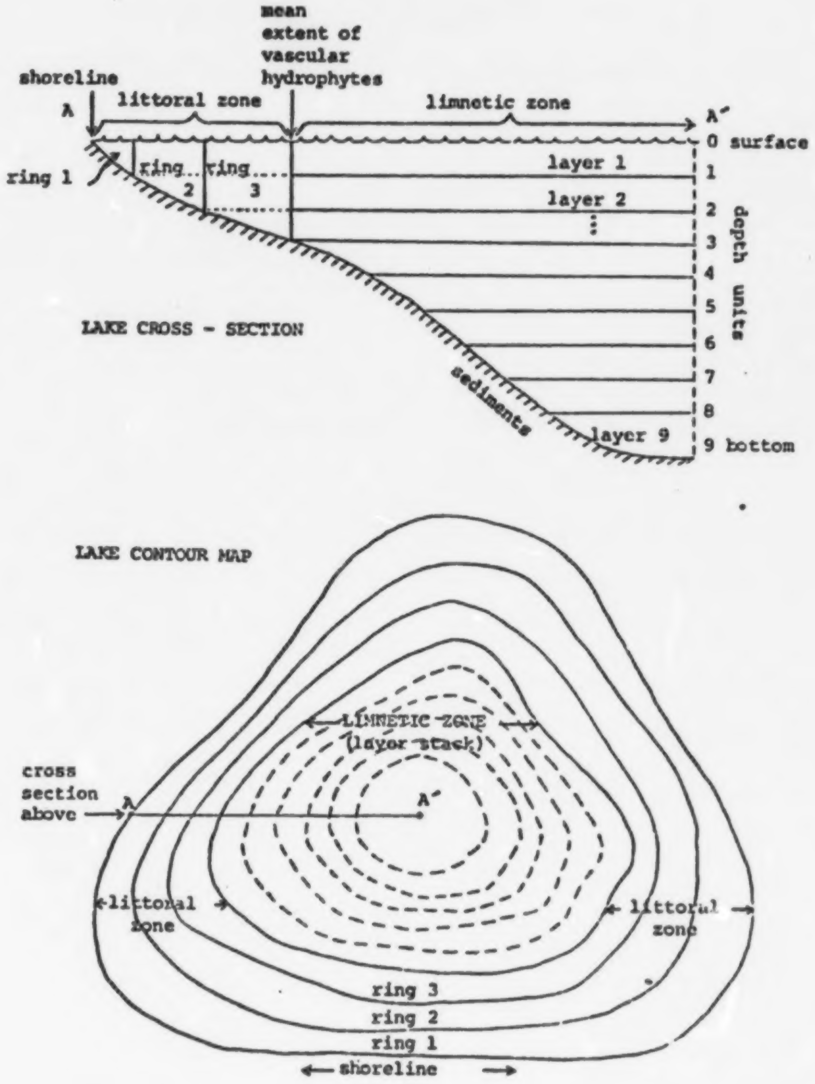
Figure 2 shows a flow diagram for a typical nutrient through the biota within each littoral zone ring. Flow rates depend on population levels and on additional variables such as pH, temperature, and light. Pesticides may directly influence the dynamics of these populations or their nutrient fluxes; conversely the organisms can alter the amounts and distributions of pesticide residues.

Figure 3 illustrates flow through the biota in a limnetic zone layer. A sample of the output from a preliminary run of the limnetic zone model for a hypothetical lake is depicted in Figure 4. Phosphorus concentration changes with depth and time in a pattern reflecting the relative complexity of component dynamics built into the model.

Parameterization is clearly a critical step in making the model useful. While parameter estimates are based largely on work reported in the literature, some parameters must be evaluated empirically during this project. Laboratory and field studies already underway include measurements of algal sinking (or flotation) rates and

Figure 1

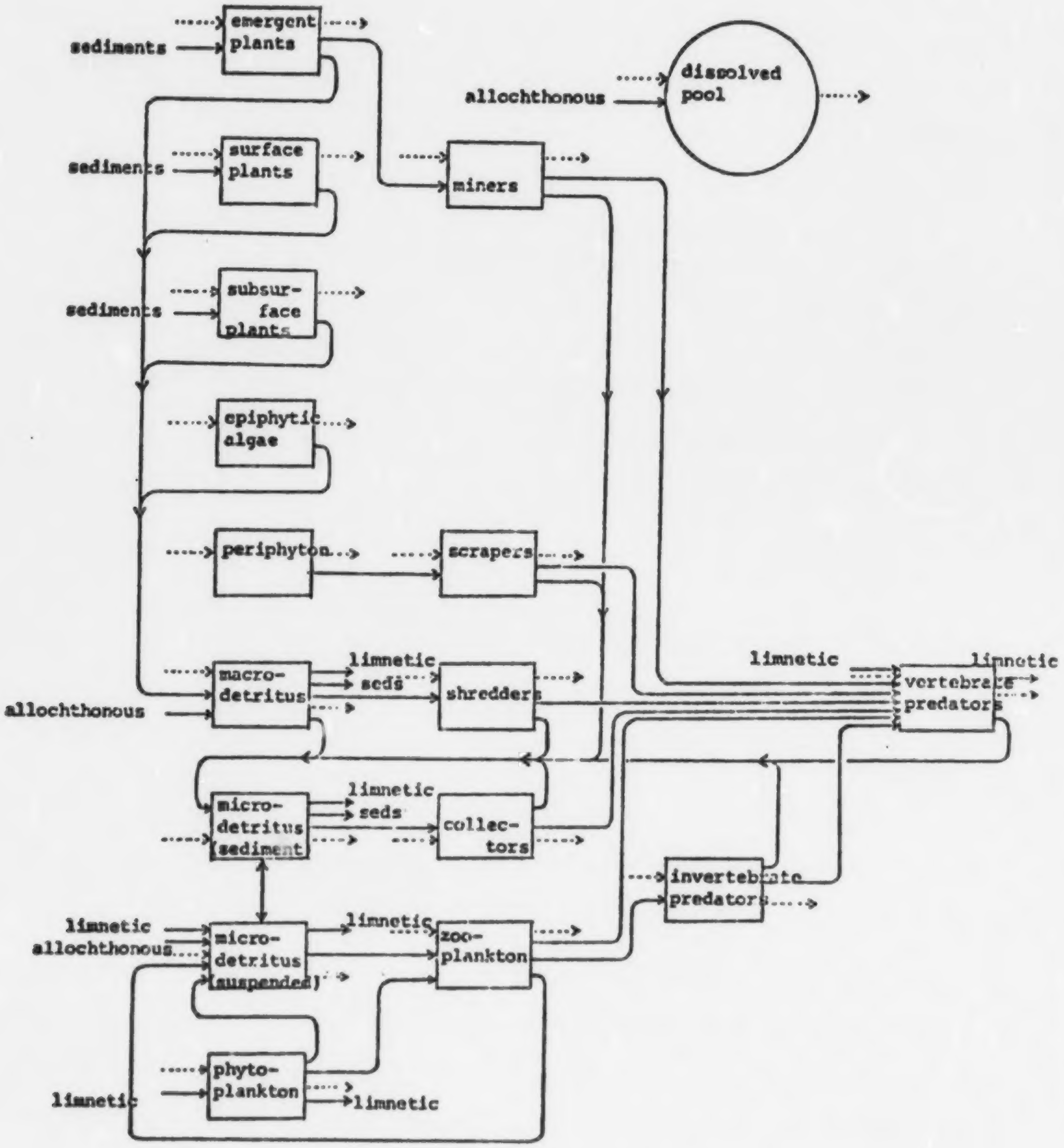
Hypothetical lake in cross-section and plan views. In this example the limnetic zone is represented by a stack of nine horizontal layers of equal thickness; uppermost layers are bounded laterally by the innermost littoral ring, and lower layers are bounded laterally by the sediments. The littoral zone is here composed of three concentric rings numbered consecutively from the shoreline to the limit of vascular hydrophytes.



mean extent of vascular hydrophytes
shoreline
A
littoral zone
limnetic zone
A'
0 surface
ring 1
ring 2
ring 3
layer 1
layer 2
layer 9
1
2
3
4
5
6
7
8
9 bottom
depth units
LAKE CROSS - SECTION
sediments
LAKE CONTOUR MAP
LIMNETIC ZONE (layer stack)
cross section above
A
A'
littoral zone
littoral zone
ring 3
ring 2
ring 1
shoreline

Figure 2

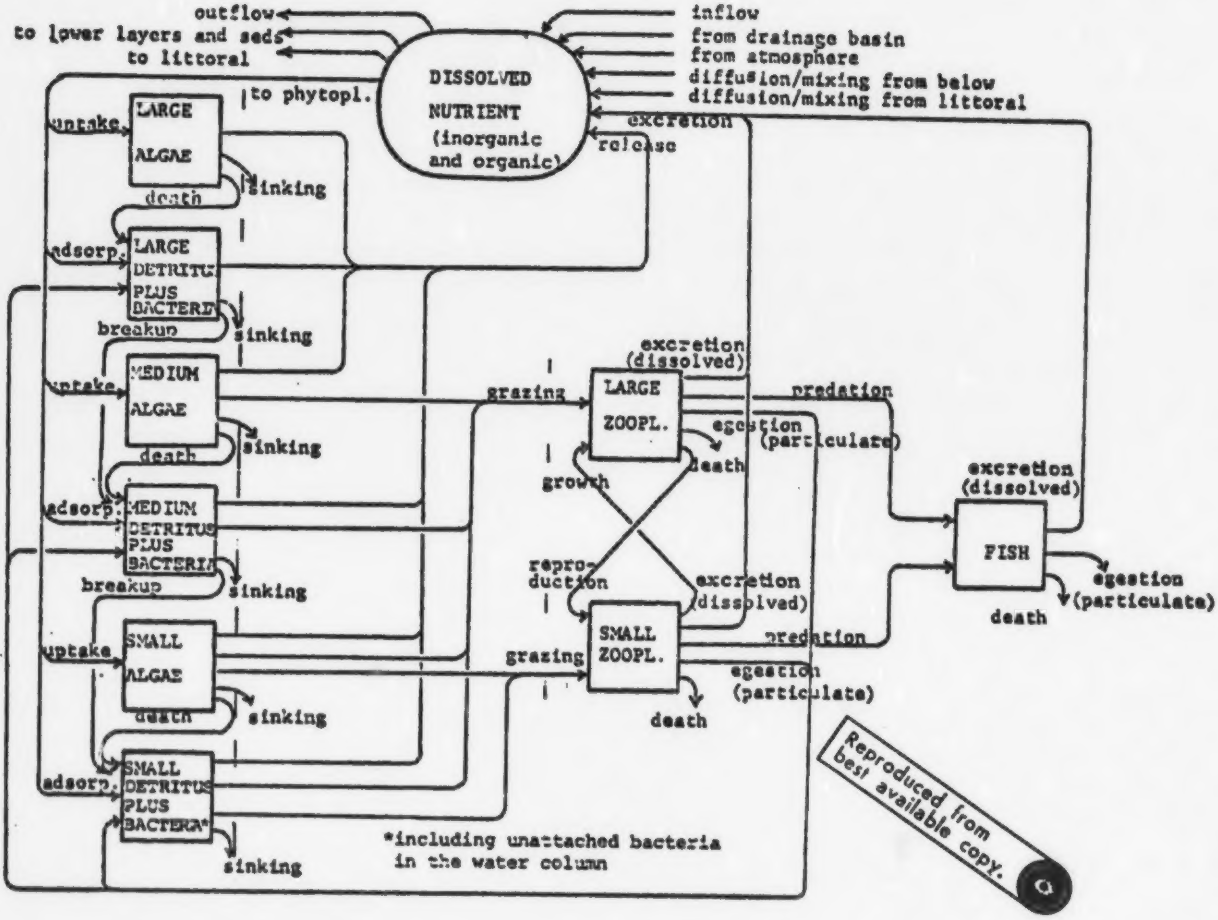
Trophic structure and the flow of organic carbon, phosphorus, and other nutrients (ignoring possible gas phases) in a littoral zone. Exchanges of each functional group with the dissolved pool are depicted by dotted arrows.



emergent plants
sediments
dissolved pool
allochthonous
surface plants
sediments
miners
subsurface plants
sediments
epiphytic algae
periphyton
scrapers
macro-detritus
limnetic
seds
allochthonous
shredders
limnetic
limnetic
vertebrate predators
micro-detritus (sediment)
limnetic
seds
collectors
invertebrate predators
limnetic
allochthonous
micro-detritus (suspended)
limnetic
zoo-plankton
phyto-plankton
limnetic
limnetic

Figure 3

Cycling of phosphorus (or other nutrient with no gas phase) in the limnetic zone. Zooplankton size categories are based on susceptibility to fish predation; phytoplankton size categories are based on susceptibility to grazing.


outflow
to lower layers and seds
to littoral
inflow
from drainage basin
from atmosphere
diffusion/mixing from below
diffusion/mixing from littoral
DISSOLVED NUTRIENT (inorganic and organic)
to phytopl.
excretion
release
uptake
LARGE ALGAE
sinking
death
adsorp.
LARGE DETRITUS PLUS BACTERIA
breakup
sinking
uptake
MEDIUM ALGAE
sinking
death
adsorp.
MEDIUM DETRITUS PLUS BACTERIA
breakup
sinking
uptake
SMALL ALGAE
sinking
death
adsorp.
SMALL DETRITUS PLUS BACTERIA*
sinking
excretion (dissolved)
grazing
LARGE ZOOPL.
predation
egestion (particulate)
death
growth
reproduction
excretion (dissolved)
SMALL ZOOPL.
grazing
predation
egestion (particulate)
death
excretion (dissolved)
FISH
egestion (particulate)
death
*including unattached bacteria in the water column

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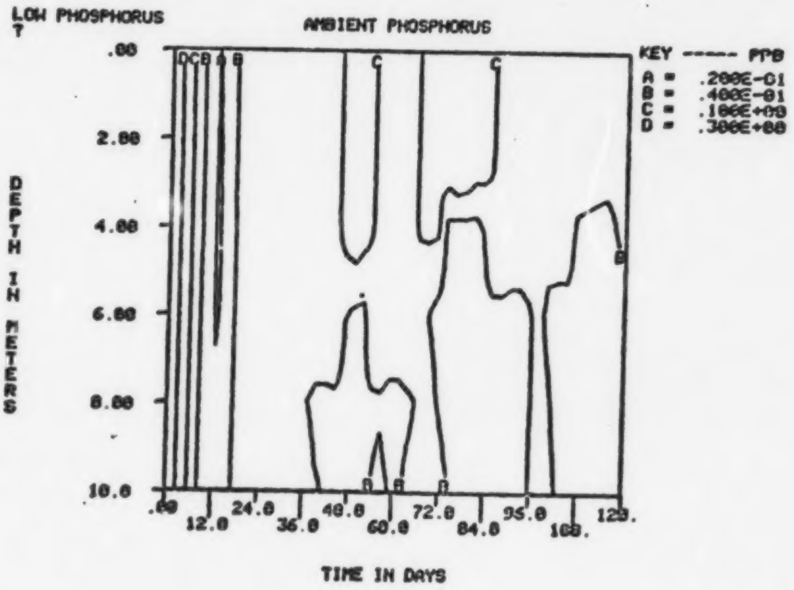
experiments on zooplankton population dynamics; studies to begin within the next month include 1) pH and inorganic carbon effects on plankton seasonal dynamics, 2) pesticide effects on periphyton in artificial stream channels, and 3) growth rates of periphyton and phytoplankton as functions of light, temperature, pesticide, and nutrients.

Figure 4

Simulation depth profiles of ambient phosphorus concentration (ppb) from April 8 through August 6 in a hypothetical cylindrical lake with low phosphorus loading.



While the aquatic impact modeling builds upon a fairly extensive literature and an earlier lake modeling program, the terrestrial impact work must begin by developing the same type of structured representation for a terrestrial ecosystem that is shown for the aquatic system in Figures 1 through 3. The initial focus will be on orchard and forest ecosystems, because they have relatively undisturbed flora and fauna (compared to tilled systems), because they are exposed to significant inputs of pesticides and have shown undesirable responses (1), and because of the experience of our research team in studying impacts of persistent pesticides on a forest ecosystem. At a later stage, attention will be given to tilled crop ecosystems such as those of corn and cotton. Concurrent studies also at Michigan State University will be particularly valuable in evaluating pesticide runoff to streams and lakes. Further stream pollution modeling studies are being proposed at Michigan State, which would help in the conceptual linking of the pesticide impacts on terrestrial and aquatic ecosystems.

Validation of the models developed will be undertaken when the parameterization activities are completed. These studies will include both observational and experimental (manipulative) comparisons. The residues resulting from routine pesticide applications in normal practice will be monitored at specific sites, and the effects on species numbers (or biomass) and their residue levels will be compared with the predictions of the model. In addition, it is expected that a limited number of small-scale direct pesticide applications to aquatic and terrestrial sites will be made in order to quantify the responses of the ecosystem for comparison with model outputs.

In summary, the research program is in the process of shifting its focus from strictly aquatic ecosystem studies toward studies of terrestrial ecosystems, with the goal of producing realistic and reasonably general models to help predict alternative pesticide impacts.

Reference

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QUESTION: I'd like to know whether your mathematical models will be deterministic or stochastic, suggestive or predictive?

DR. GOODMAN: They are deterministic and predictive. We're not trying to build into our functions any probability distributions and look at them from that point of view. We will be accomplishing a similar thing by doing high-low level sensitivity analysis--that is, putting in high and low values for variables and determining what happens. We'll be trying to bound the responses that we might get. That doesn't give us exactly the same type of information that you'd get with a stochastic model; however, it reduces the model one level of complexity.

QUESTION: What mathematical form are you going to be using in your model -- differential equations, difference equations, or what?

DR. GOODMAN: We'll be using difference equations, and at this point for our aquatic models we're using a time step of a tenth of a day in determining rates. Obviously this requires higher level representations for some rates than would otherwise be used, and that requires our performing some calculations relatively often. We're trying to economize wherever we can though, by choosing an appropriate time interval for various processes.

QUESTION: The rates of accumulation, concentration and dissipation-- since these are continuous constants, how are you going to describe them in mathematical terms?

DR. GOODMAN: We have to integrate over short time periods in order to translate from a continuous process into a discrete process; in digital computer simulation we're limited to dealing with discrete processes. But the methodology for translating from continuous time processes to discrete time processes is fairly well developed and we're using that methodology. We'll be parameterizing the system, however, as difference equations rather than strictly differential equations.

QUESTION: Are you using "systems analysis" in the sense of contemporary engineering, or input-output analysis, or what methodology?

DR. GOODMAN: We're not doing input-output analysis. We're using discrete time state-space systems theory such as is employed generally in engineering studies of control theory problems.

QUESTION: Is your model a distributed lag model?

DR. GOODMAN: It is not explicitly written as a distributed lag model. There are, however, for many populations, multiple life stages which essentially yield lags in the model. Thus, you could analyze it as distributed lag model, but that's not the particular methodology from which we're trying to build it.

QUESTION: You have a framework there that you showed us to describe nutrient dynamics in the lake, and I wondered how you're going to go about tying the pesticide in there with that?

DR. GOODMAN: For each organism that we represent and each of the major processes that may be involved in transfer of biomass among organisms, we will be trying to determine for particular individual pesticides how they accumulate in the organisms, if they do; what mortality they exact, if they do; and then how they influence rates in other fashions. We expect to see some more or less direct effects which will depend, of course, very heavily on the concentrations that we're looking at, the range we're looking at.

We also expect to see some indirect effects; that is, effects which are not directly caused by the pesticide but are mediated by the perturbations you've caused in populations. If you wipe out all of one species, you're going to change the trophic dynamics of the system and we have to try to put some bounds -- this is really the goal -- on what those effects may be and how they may proliferate through the system. Can you set up unstable oscillations? Can you cause effects of this sort on the ecosystem?

QUESTION: Sir, you've described a full system here. If I understand it right, based on the diagram that you showed, it would be a fairly detailed, almost microscopic view of many aspects of the system, in order to include every aspect to consider and relevant to a whole system, rather than a macroscopic view of the system. Is that correct?

DR. GOODMAN: I think our notion is that we don't want to get any more microscopic than we have to, but where we see a process that is influenced by the dynamics of the pesticide itself, we do have to break it up. That's why, for example, we're hung up on the algal dynamics -- because of their tremendous potential for cleaning things up or passing them up to higher trophic levels. We plan to do much less detailed models of higher elements, even though they are integrative in nature.

QUESTION: Have you perchance done any rough calculations on the number of parameters you will want to do sensitivity analyses for?

DR. GOODMAN: We've sat down and looked at that kind of thing and obviously we're not going to be able to do total, global sensitivity analyses on all of these parameters. Many of these will be done on chunks of the model -- on components -- and then larger behavioral characteristics will be assembled to get the total system behavior.

DR. GLASS: One thing Erik didn't mention in his talk is they've been developing this model -- what? Two, three years under NSF funding?

DR. GOODMAN: Well, one year under funding and two years with no funding.

MATHEMATICAL MODELING OF PESTICIDE FATE*

Dr. James W. Gillett**

Those of you who have had an opportunity to meet Jim Haefner and know him will appreciate the humility with which I present his work. Unfortunately, he was with us only seven months, and I wish it could have been much longer. The difficulty of present whole ecosystem mathematical models has just been illustrated by Dr. Goodman's work. Our problem is in some ways simpler and in some ways more difficult, to try to represent mathematically the disposition of a pesticide within a terrarium. Fortunately, we have some good help on this, especially those people at Oregon State University, who are deeply involved in similar work, and those at Athens Research Lab: Jim Hill, Jim Falco, Ray Lassiter, and George Bailey. Discussions on the Pesticide Runoff and Transport Model (Bailey et al., 1973) have been very useful. Unfortunately, their system is not applicable to our model because the intensity of interactions within a microcosm changes the scope of work.

In most systems — I think Dr. Kenaga would support me on this — the mass deposition of a pesticide is not greatly involved with the biota. The disposition within the biota may become critical in relation to the total biotic structure. Where the mass of the pesticide goes is not affected as much by the biota, as by the media. Within a microcosm, within the confines of an intense, closed system, the biota become very critical. Thus we have two sets of processes on which we are working as parts of the model. The first set is the so-called pesticide transport process (PTP); the second set is the biological control processes (BCP). These latter processes with non-pesticidal inputs and outputs regulate functions which cross over to pesticide transport processes and which receive inputs from pesticide transport processes (Figure 1).

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*Co-author is James Haefner

**National Ecological Research Laboratory, Corvallis Environmental Research Laboratory, Office of Research and Development, EPA, Corvallis, Oregon

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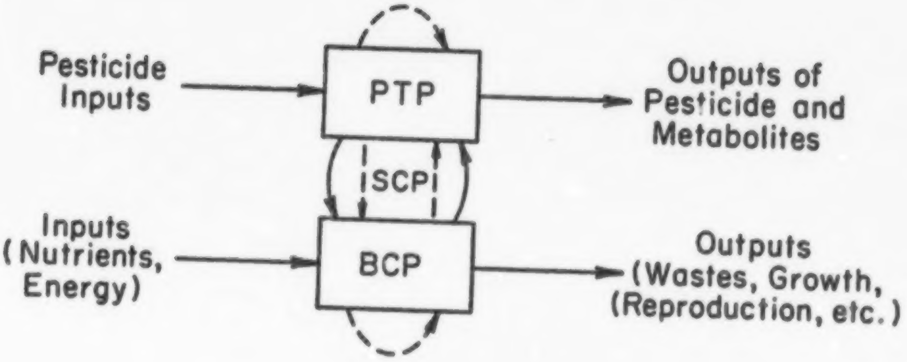
Figure 1


Pesticide Inputs
PTP
Outputs of Pesticide and Metabolites
SCP
Inputs (Nutrients, Energy)
BCP
Outputs (Wastes, Growth, (Reproduction, etc.)

In the real world it is likely that the PTP box is very large and the BCP box is very small. In our microcosm we suspect that they are much more equal in size and, hence, much more consequence can be attributed to the interactions between the two. Those arrows between the boxes constitute the system control process (SCP). We view this system control as a means of predicting the disposition of a pesticide within the terrarium. The purpose of doing the mathematical model is not to provide a means of predicting the disposition of the pesticide in the real environment, in a total ecosystem or any realistic segment of an ecosystem, such as a crop land. It is to provide us with a means of analyzing the structure of our terrarium and the results from it. We hope to understand the processes in the terrarium and in the model using this tool.

A number of mathematical models have provided input into our concepts. The Stanford hydrology model and the subsequent Wisconsin hydrology models, which led to the PRT, are an order of magnitude or greater in scale, so that they are not directly applicable. Also, the work done on soil columns, the very elegant work of Lindstrom, Boersma and Hall, the work of Lichtenstein's group, and the work done at NERL in Athens also are not directly applicable. These are based on very hyper-fine processes, highly resolved and relatively well understood, but they have no direct relationship to the larger world involving biological processes and movement.

The part of the system which is least understood of all in which we are involved is the below-ground ecosystem. It is very difficult to work in, partly because we cannot observe it in the sense that we can observe fish in an aquarium. We have difficulty sampling it and difficulty constructing it. That is, we cannot make a soil the way we can make artificial seawater, brackish water, or fresh water.

In addition to the differences in the kinds of matter that are in soil, we do know that the differences between the types of dissolved organic carbon and the types of particulate matter have great consequences on the function of that soil system. These functions are affected by such potent environmental forces as temperature, moisture, and cation availability. The controllers of these processes may be microorganisms or macroorganisms (mites — the shredders; earthworms — the vertical movers and distributors) which change the spatial, geometric distribution of these materials and thus their biological availability within the system.

Using McBrayer's model in general, Dr. Haefner has constructed a similar model (Figure 2) which reflects most of the processes with which we are concerned, not

Figure 2

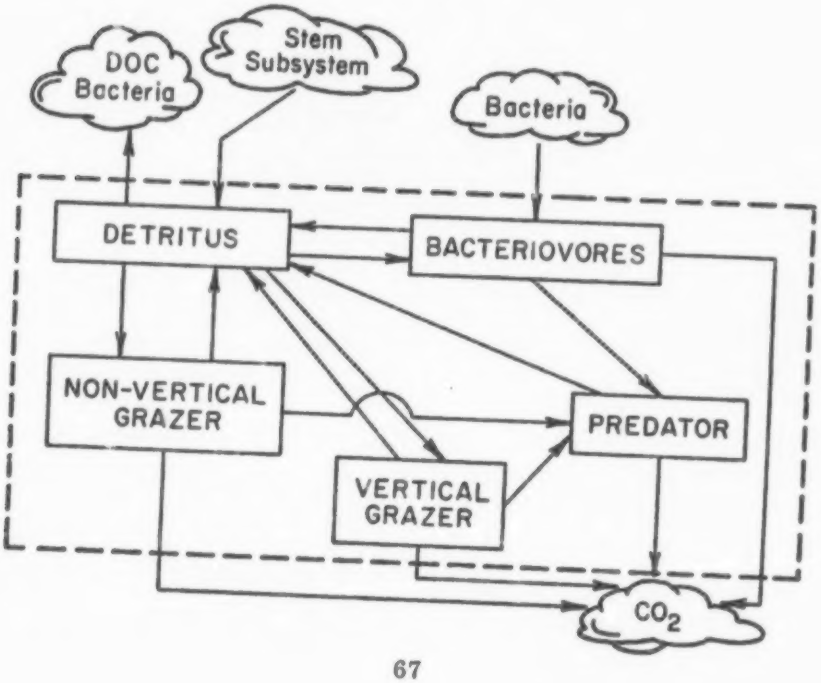
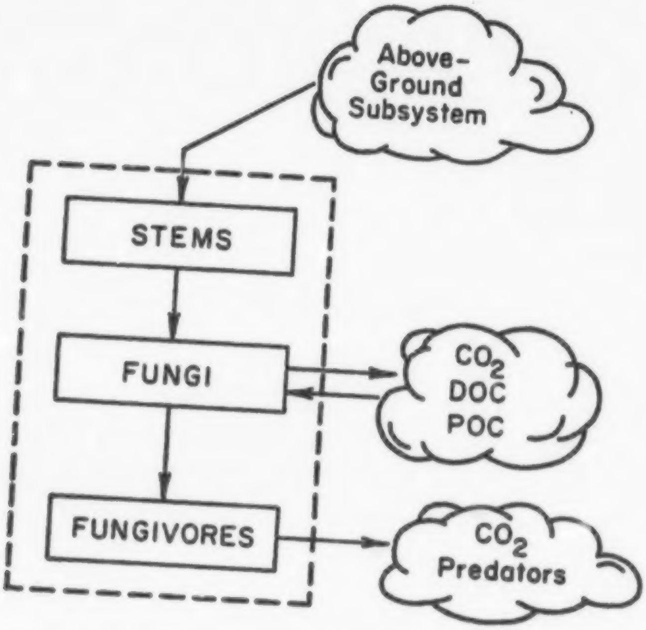
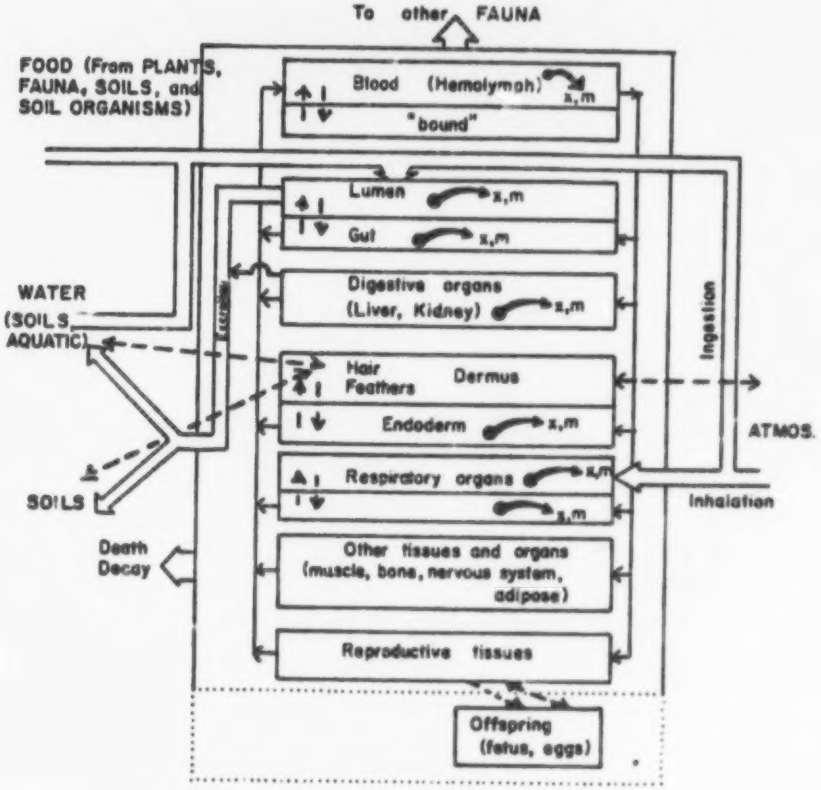


Figure 3



just in our terrestrial microcosm for pesticide studies, but also in microcosms for litter decomposition involved in evaluation of coal-fired power plant siting. Particulate organic carbon divided into arbitrary size classes can be handled either stochastically or strictly on a class basis. The movement of animal and plant organic carbon, feces, carcasses, exoskeletons on one hand, and leaves, stems, and root exfoliates on the other into dissolved organic carbon is extremely critical. Similarly, we need to look at the fungal breakdown, particularly of the woodier materials (e.g., stems) (Figure 3). The relationships between the fungi and fungivores and between the bacteria and bacteriovores are very complex and not well understood. However, there may be means of discriminating between these different functions, using certain optical techniques that Dr. Bruce Lightheart has been working on in NERL that will allow us to discriminate between live and dead bacteria and fungi, which in turn will allow us to make some estimates of active biomass. All of the optical techniques and chemical techniques in this area suffer in some regard, however.

Figure 4


To other FAUNA
FOOD (From PLANTS, FAUNA, SOILS, and SOIL ORGANISMS)
Blood (Hemolymph)
x,m
"bound"
Lumen
x,m
Gut
x,m
Digestive organs (Liver, Kidney)
x,m
WATER (SOILS, AQUATIC)
Ingestion
Hair Feathers
Dermus
Endoderm
x,m
ATMOS.
Respiratory organs
x,m
x,m
SOILS
Inhalation
Death Decay
Other tissues and organs (muscle, bone, nervous system, adipose)
Reproductive tissues
Offspring (fetus, eggs)

In the higher organisms it would be possible to develop a pharmacokinetic model (Figure 4) of the fate of the pesticide. Since we are most interested in the vertebrate predator in the system, we may develop a model such as has been developed for dieldrin (Lindstrom et al., 1974).

In the above-ground ecosystem, the International Biome has created a series of grassland models and other models which are very useful. These systems are well enough established that we will not need to invest as much time in that area. The mathematical models are quite elegant and very easily obtained.

Figure 5

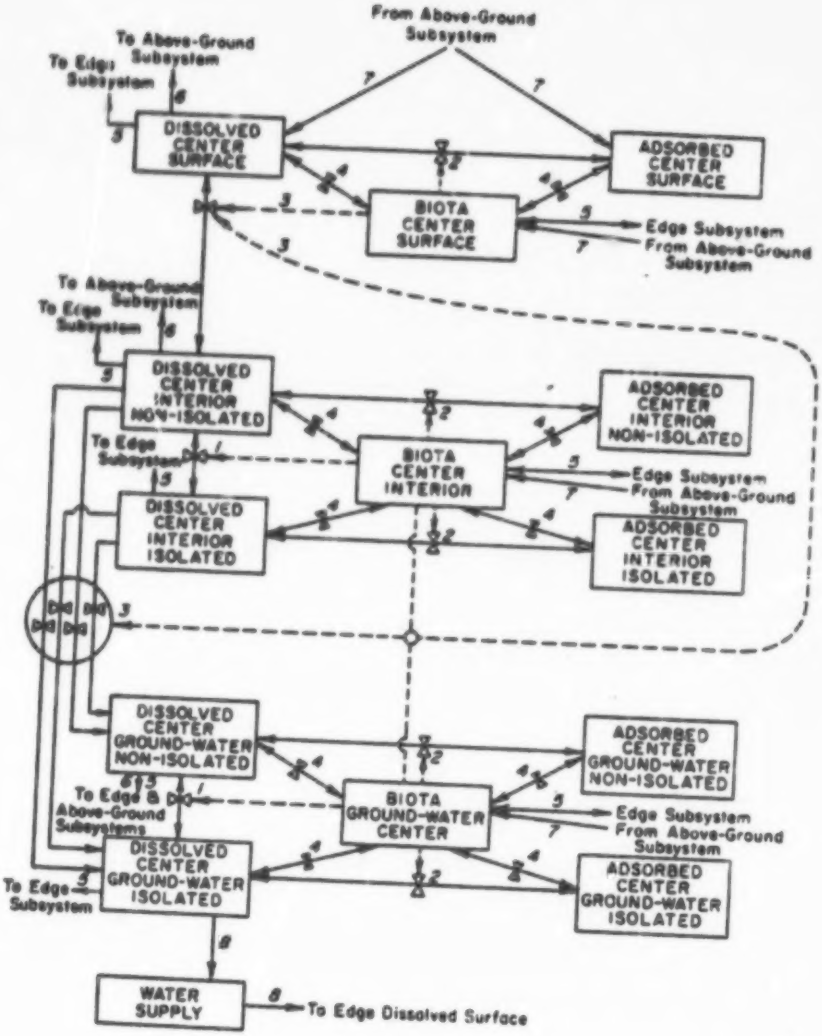

From Above-Ground Subsystem
To Above-Ground Subsystem
To Edge Subsystem
DISSOLVED CENTER SURFACE
ADSORBED CENTER SURFACE
BIOTA CENTER SURFACE
Edge Subsystem
From Above-Ground Subsystem
To Above-Ground Subsystem
To Edge Subsystem
DISSOLVED CENTER INTERIOR NON-ISOLATED
ADSORBED CENTER INTERIOR NON-ISOLATED
To Edge Subsystem
BIOTA CENTER INTERIOR
Edge Subsystem
From Above-Ground Subsystem
DISSOLVED CENTER INTERIOR ISOLATED
ADSORBED CENTER INTERIOR ISOLATED
DISSOLVED CENTER GROUND-WATER NON-ISOLATED
ADSORBED CENTER GROUND-WATER NON-ISOLATED
To Edge & Above-Ground Subsystems
BIOTA GROUND-WATER CENTER
Edge Subsystem
From Above-Ground Subsystem
To Edge Subsystem
DISSOLVED CENTER GROUND-WATER ISOLATED
ADSORBED CENTER GROUND-WATER ISOLATED
WATER SUPPLY
To Edge Dissolved Surface
1
2
3
4
5
6
7
8

Figure 5 illustrates the consequences of the biological control and pesticide transport processes interaction, the control process. The terrarium has an "edge" and a "center." Nematode concentrations at the edge may be two orders of magnitude greater because of greater moisture content and particulate organic matter, providing for greater bacterial growth. In addition to the lateral zones, the model has vertical zones: "center above-ground," "surface," "soil intermediate" and "groundwater."

A new concept being explored is that of "isolated" and "non-isolated" soil. "Non-isolated" soil is interacting directly with the atmosphere; "isolated" soil is not interacting with the atmosphere. The proportion of isolated soil may be temporal related to the changes in moisture and heat content and to biologic activity. The concept of the non-isolated and isolated soil may be a useful approach to assessing volatilization and movement of materials from the soil.

These compartments of the model represent concentrations for the parent chemical. For each metabolite or breakdown product, a similar, parallel model is assumed. The difficulty that has not been surmounted by any modeler to date involves modeling multiple inputs of the metabolite from a given chemical into a parallel model of the metabolite. It would appear to be mathematically feasible, although elaborate and frought with unknowns. In a number of instances, however, it is the metabolite in which we are interested and not the parent compound. While one has hope for deriving a mathematical model of the disposition of a pesticide, the problem of integrating the metabolites with the parent compound would seem to be a serious difficulty in the future development of terrestrial microcosm models.

EFFECTS OF PESTICIDE USE PATTERNS ON THE INCIDENCE OF PLANT DISEASE AND ON PATTERNS OF PESTICIDE DEGRADATION

John Bowser*

This research program will be conducted under an interagency agreement between EPA and USDA. Funding for the agreement will come from the Substitute Chemical Program and USDA. Since the work is just getting started at this time, there are no experimental results to present.

I will discuss the need for the research and why it's being funded under the Substitute Chemical Program. I will also outline what we hope to accomplish through this work, how we plan to conduct the experiments, and why the work is being done in cooperation with USDA.

The work to be performed under this agreement deals with two separate but interrelated problems. The first is the potential for alteration in rates of incidence of plant disease in crops which have been treated with pesticides. The second problem involves possible alterations in pesticide degradation patterns which can result from the use of various pesticide combinations.

I'll first discuss the work concerning the effect of pesticides upon the incidence of plant disease. A number of reports have appeared in the literature which indicate that in some instances use of certain pesticides may result in change in the incidence of certain plant diseases. These reports include descriptions of instances in which severity of plant disease was increased (1, 4, 5, 6), as well as instances in which the rate of incidence of plant disease decreased concurrent to the use of certain pesticides (2, 3, 5, 6).

These findings are of interest to us since, in order to carry out the Substitute Chemical Program properly, we must be able to anticipate what will

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* Office of Pesticide Programs, Criteria and Evaluation Division, EPA

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happen if one pesticide is substituted for another. We must have a way to predict whether the adoption of a new pesticide use pattern will result in a change in plant disease patterns faced by the pesticide user. That is, will the user suffer greater or lesser losses due to plant disease, or will there simply be no change observed in the incidence of plant disease?

We found the current literature lacking in regard to this problem. First of all, it is sparse. For many of the compounds to be evaluated under the Substitute Chemical Program, there is no information of this type. Secondly, the studies which are available are generally laboratory or greenhouse studies which have not been confirmed by field observations; therefore, their value in determining the results which will be experienced by the user is unclear. Thirdly, the possible effects of pesticide combinations in this area have not been examined; that is, the studies which have been conducted have been on single compounds, not on combinations of compounds.

In view of the problems which I have outlined above, we have designed a research program which is intended to meet the following goals. First, we would like to fill some of the data gaps in regard to the chemicals we're interested in under the Substitute Chemical Program. Secondly, we'd like to conduct experiments under both greenhouse and field conditions so that we'll be able to correlate results observed in the greenhouse with results observed in the field. Finally, we'd like to demonstrate a relatively simple and inexpensive procedure which can be followed by other researchers in the future.

The initial work will be laboratory or greenhouse studies of 20 to 25 pesticides or combinations of pesticides. These studies will include investigations of the incidence of plant disease, the effects of the pesticides on antagonists to the particular pathogens under study, and the effects of the pesticides on microbial activities which are normally associated with maintenance of soil fertility. The lab and greenhouse studies will be followed by studies of four or five pesticides or pesticide combinations under field conditions.

The crop-pathogen combinations, as well as some of the pesticides, which have been selected for these studies are listed in Tables 1 through 3.

Table 1

Crop:	Snapbeans	
Pathogen:	*Rhizoctonia solani*	
Herbicides:	Dinitroaniline (trifluralin)	Plus phenolic dinoseb
		Plus thiocarbamate (EPTC)
	Thiocarbamate (EPTC)	Plus phenolic dinoseb

Table 2

Crop:	Soybeans	
Pathogen:	*Phytophthora megasperma*	
Herbicides:	Phenolic dinoseb	Plus aniline deriv. (alachlor)
		Plus benzoic acid deriv. (chloramben)
		Plus dinitroaniline (trifluralin)
	Dinitroaniline (trifluralin)	Plus triazine (metribuzin)

Table 3

Crop:	Cotton	
Pathogen:	*Thielaviopsis basicola*	
Herbicides:	Phthalate deriv.	(DCPA)
	Thiophosphate	(bensulide)
	Urea	(fluometuron)
	Triazine	(prometryn)
	Aniline deriv.	(alachlor)
	Phenolic	(DNBP)
	Dinitroaniline	(trifluralin) plus phenolic DNBP
	Dinitroaniline	(profluralin)
	Thiocarbamate	(EPTC)

As you can see, we have decided to concentrate on pre-emergence herbicides in this study. The herbicides will be applied at rates consistent with normal agricultural practice. Furthermore, we've made an attempt in the selection of these pesticides to include representatives from the major chemical families.

Dr. George Papavizas and Dr. Jack Lewis of the USDA's Soilborne Diseases Laboratory will be the USDA project officers for this portion of the agreement.

Dr. Donald Kaufman of the USDA's Pesticide Degradation Laboratory will be the project officer responsible for studies to be conducted under the second half of this agreement. The work will be designed to detect changes in the patterns of degradation of various types of pesticides which occur when these pesticides are used in combination with other pesticides.

Dr. Kaufman's work will involve initial laboratory screening of 20 pesticide combinations. This initial work will then be followed by field tests on four or five pesticide combinations. The compounds used for this work will include representatives of major pesticide chemical families.

The USDA was selected to carry out this research primarily because of the excellent combination of expertise and facilities available at the Agricultural Research Center at Beltsville, Maryland. A contributing factor in our decision was the USDA's willingness to assume a portion of the financial costs of the project.

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QUESTION: Which factors are you going to examine in your soil fertility studies?

MR. BOWSER: We'll be concentrating on microbial activities such as ammonification and nitrification. We feel that these indicators are more valuable than simple estimates of microbial populations in the soil.

BENCHMARK CHEMISTRY PROGRAM

Dr. Riz Haque*

I would like to point out that this program has just been started, so I don't have any scientific results to report, but I will describe the rationale and conceptual scheme behind this program. I shall begin with Regulation Three, the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, which states that a pesticide, in order to be registered, must perform its intended function without unreasonable adverse effects on man or the environment.

This is one of the most important regulations as far as the registration of pesticides is concerned. During 1974, under appropriation PL 93-135, the Substitute Chemical Program was started. The objective of this Program is to find substitutes for problematic pesticides. The registration of pesticides or the finding of substitutes for undesirable or problematic pesticides requires a critical evaluation of data from various disciplines and the establishment of the effects of pesticides on man and environment. Some of the factors used in the evaluation of pesticides are 1) physical and chemical properties, such as water solubility, vapor pressure, melting point, boiling point, and structure; 2) toxicology -- we have to know about acute and chronic toxicity; 3) metabolism -- what the metabolites of the particular pesticides in soil, plants, or animals are; and 4) fate and behavior -- the transporting soil, bicaccumulation, residues in water, air, and soil.

However, the problem of defining an unreasonable adverse effect on the environment is extremely difficult. Furthermore, other factors, such as the mere number of pesticides and metabolites and the existence of various formulations for any particular pesticide, make this task very tedious and cumbersome. The introduction of simulated modeling techniques has given a new approach to the study of behavior of pesticides in the environment; however, we still do not have any practical model.

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*Office of Pesticide Programs, Criteria and Evaluation Division, EPA

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One of the most important questions which we are faced with now is whether we can somehow simplify the evaluation process for pesticides. The purpose of this benchmark chemistry program is to qualitatively predict the behavior of a pesticide from a knowledge of the chemodynamic parameters of selected benchmark pesticides.

The words benchmark and chemodynamic are relatively new, so I would like to define these terms. Benchmark is the name given to a group of pesticides selected from each important class, based on the structure and use pattern. The idea behind benchmarks is that if we know the behavior or properties of one or two pesticides from each important class, then we can predict the behavior of any chemical on a qualitative basis. I would like to point out that this list (Table 1) is just selected randomly, on the basis of structure, and there is no special reason for any particular compound to be included. We hope that if you know two or three chemicals from each class, then you can predict the behavior of others from that class.

Table 1: Suggested Benchmark Pesticides

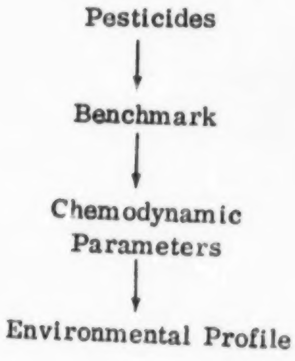
Chemical Class	Pesticide
Aliphatic Acids	Dalapon
Aniline-Based	Chloropropham, Alachlor
Benzoic Acids	Dicamba, Chloramben
Carbamates	Carbaryl, Carbofuran, Methomyl
Dinitroanilines	Trifluralin
Fumigants	DBCP; Methyl Bromide
Organochlorines	Lindane, Dieldrin, Methoxychlor, Mirex
Organometallics	MSMA, Methyl Mercury, Tributyltin
Organophosphates	Malathion, Diazinon, Methyl Parathion, Phorate, Fensulfothion Chlorpyrifos, DDVP, Leptophos
Phenols	Dinoseb, PCP
Phenoxyalkanoic Acids	2,4-D; 2,4,5-T
Phthalamides	Captan
Quaternary Salt and Picolinic Acid	Picloram, Paraquat
Thiocarbamates	EPTC
Triazines	Atrazine, Simazine
Ureas	Diuron, Monuron
Miscellaneous	Dichlobenil, Benomyl, Altosid, Bifenox, Bromacil
Natural Pesticides	Pyrethrums
Aromatic Nitro Compounds	PCNB

As you see, this represents aliphatic acids, carbamates, dinitroanilines, organophosphates, organometallics, phenols, triazines, ureas, and so on. If we take into account the structure and use pattern, these may represent 80 percent of the pesticides used in the United States.

Chemodynamics is a field which describes the use of physical chemical properties and the processes associated with them in predicting the behavior of a chemical in various parts of the environment. For example, if we want to predict the behavior of a chemical in the air, then we should know the vapor pressure and vapor loss. If we want to know the behavior in water, we should know solubility, heat of solution, and half-life for hydrolysis. Similarly, for soil we have to know adsorption/desorption, leaching, and degradation. For biota we measure the octanol/water partition coefficient or fish bioaccumulation. Then in order to account for the total degradation we study photodegradation. These parameters can give us a rough estimate of the physical as well as biological behavior of pesticides.

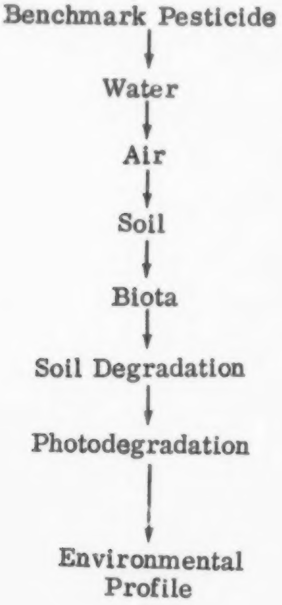
Once we have the parameters, we put them in a matrix form and try to build up an environmental profile. From this profile we can predict the behavior of each class of pesticides. Figure 1 is a schematic diagram of how we screen a pesticide through this benchmark program. We have a large number of pesticides from which we select the benchmarks. On the benchmarks we carry out the chemodynamic studies and then build up environmental profiles.

Figure 1



In Figure 2 we have the various chemodynamic parameters, such as water, air, soil, biota, soil degradation, and photodegradation, from which we build up the profile. The idea behind the benchmark program is to obtain the benchmarks, measure the chemodynamic parameters, put them in the matrix form, and then, from the principle of pattern recognition, build up a stereotype profile for each class of compounds.

Figure 2



This program is divided into six parts, and it is expected that the work on each part will be carried out in different labs. It's very unlikely that we could find one lab which is fully equipped and has expertise in all these areas. For the air work, we will measure the vapor pressure, vapor loss from soils, and effect of temperature and moisture on vapor loss. For the water part, we will measure solubility, dissociation constant, hydrolysis, effect of temperature, and pH. For soils, we will measure the adsorption, desorption, leaching, effects of temperature and moisture, and pH. For biota, we will measure the octanol/water partition coefficient and fish bioaccumulation.

It has been shown by Dow scientists that the octanol/water partition coefficient could be used as a guide for accumulation of pesticides. We propose that, if you measure the octanol/water partition coefficient, you can tell the degree of accumulation of the pesticide in a living system.

For soil degradation we measure the rate of degradation in soils and identification of metabolites. In photodegradation we study the effect of sunlight and also the identification of products. These are the various factors which are involved in the benchmark concept, and we expect that in each part we'll be studying the same pesticides under similar environmental conditions, so that results obtained from one part will be comparable to results from the others. We also hope to study the same number of pesticides and similar pesticides, so that we won't have a gap when we try to put all the parts of the jigsaw puzzle together to get the profile.

Secondly, we should use the same grade of pesticides. This is necessary in order to observe any surprising effect of the impurities. Third, similar analytical methods should be used as far as possible. These may include spectroscopic techniques, GLC, and radioactive tracer methods. Fourth, quality assurance -- we should make sure that all the results have similar analytical precision. Fifth, the studies should be carried out with active ingredients as well as technical formulations. In the literature, most of the work on pesticides has been done with active ingredients; whereas we use pesticides in field as formulations, either as wettable powder or dust. Therefore, we should make sure that by changing from a pure chemical to a formulation these parameters are not affected significantly. Finally, we need good communication -- among all these parts we expect that there'll be open exchange of information.

The benchmark chemistry program is going to be divided into two phases. Phase one, which involves the evaluation of literature, is being carried out in cooperation with Ms. Pulliam, and this work has been contracted to George Washington University. We expect the work to be completed shortly.

With George Washington University we have identified several experts in this country who are especially suited to carry out the work. As consultants, they are helping George Washington University evaluate the literature work. Dr. Spencer is supplying information on air work; Dr. Freed of Oregon State University, on water; Dr. Farmer of University of California, Riverside, on soil adsorption; Dr. Metcalf of the University of Illinois, on bioaccumulation and octanol/water partitioning; Dr. Kaufman of USDA, Beltsville, Maryland, on soil degradation; and Dr. Crosby of University of California, Davis, on photodegradation.

Phase two is the actual lab work, which is barely underway. Only two parts, the air part and soil degradation part, have been signed under interagency agreement with USDA. Dr. Spencer is carrying out the vapor pressure determination and vapor loss studies, while Dr. Kaufman is carrying out soil degradation studies, including the identification of metabolites. The other parts -- water, soil, biota, photodegradation, and modeling -- will be starting shortly, and we expect to have some input from the Office of Research and Development and the Office of Toxic Substances.

Finally I'd like to summarize the scope and advantages of this benchmark program. Number one, it would be used in the Substitute Chemical Program. For example, if you have to find a substitute for chemical X, then from the use pattern you may find that there are a thousand or maybe several hundred chemicals available as substitutes. By comparing the profile of this compound X with all these chemicals on the benchmarks, you may be able to eliminate many of them and so you can concentrate on only a few chemicals. In this way, use of the benchmarks can simplify the Substitute Chemical Program.

Number two, benchmarks may be utilized in the registration process. For example, if you have a new chemical for registration, you are provided various data by the registrant. You can compare the profile of this chemical with the benchmarks and find out where any potential problems may exist. I must point out that this benchmark concept would not replace the data requirements but simply assist the evaluation process.

Number three, from the benchmark program we'll find out where these chemicals will concentrate in the environment. This should assist in planning long-term monitoring and toxicology studies.

Number four, this benchmark program can be applied to other toxic chemicals and, in this respect, I would like to point out that we are helping the National Science Foundation. The Foundation plans to employ this benchmark concept for other toxic chemicals.

Number five, we are going to carry out these studies on many compounds, and I'm sure we'll find some interesting research problems.

Number six, most of the experiments described in the benchmark program are lab work and are of relatively short duration, so we should get this information quickly.

And number seven is, for my own bias, simplicity.

COMMENT: After listening to this dialogue on benchmark chemicals, I don't know why we have to submit any data for registration of a compound. You plan to do it all over again, and these data have already been taken care of quite adequately.

DR. HAQUE: You have a good point there, and we have given consideration to this point. If you look in the literature, you will see that this work has been carried out in different labs, under different environmental conditions. It is very difficult to compare the results.

COMMENT: If someone wants to redo all the degradation, all the literature work, and all the experimentation we've done in the field, it's not going to be done simply. It takes a long time, in excess of six, seven years and about $8 million to get a pesticide in the marketplace, and they're not all the same.

DR. HAQUE: I would like to point out that if you have followed my talk, you will note there are two phases of this program. Phase I is the literature work.

From the literature work, if we are convinced that the data are available, then we are not going to do experimental work.

COMMENT: As far as the compound that we have that's in review by the Substitute Chemical Program, everything I saw on that slide is available.

MS. SCHUTH: There are a lot of areas where data are not available, like water solubility and vapor pressure. When available, there is standardization of measurement techniques. We're not talking about going out and repeating all your studies. We're just talking about defining five or six parameters and having them all measured in the same way, so that these values will be useful in our models and will be more valuable for comparative purposes.

QUESTION: I question one other thing. I can agree with your point on water solubility. It's 20, 25, 30, 35 degrees centigrade, and volatility under a variety of conditions, I can understand this, but I can't say that the benchmark chemical is going to satisfy and be comparable in the total series of compounds that belong in the same class. I think the literature points that out very clearly. They metabolize somewhat differently. They have different products.

DR. HAQUE: Therefore, we have more than one compound in each class, and we are saying that, if you look at, for example, chlorinated hydrocarbons, then you have the problem of bioaccumulation. We are trying to identify the problems in each class, or various characteristics of types, and we are not really going to do all the studies. There are just a few parameters we are going to be studying under exactly similar conditions. Some of the data in the literature are really not very good.

QUESTION: I wondered if you'd considered how you would approach this with reference to benchmarks when you get a compound and have several different functional groups related between classes?

DR. HAQUE: Then we may have to compare several benchmarks from the several groups, for example, one compound may fall in two or three classes. We may have to look at different classes.

DEVELOPMENT OF RAPID SCREENING TECHNIQUES FOR HERBICIDE PHYTOTOXICITY

Dr. Fumihiko Hayashi*

An important part of the Substitute Chemical Program is to select, evaluate, and test pesticides that may be used as alternates to certain registered products. Phytotoxicity of pesticides is currently identified and measured by observable symptoms which may or may not be the result of biochemical disruptions at cellular levels. Such physiological or physical injury can be easily discerned and is not the subject of this research project. Rather I would like to address the subject of how to measure invisible phytotoxicity resulting from pesticide usage.

Symptoms displayed by plants from biochemical disruptions are results of biochemical stimuli at cellular levels and may result in alterations of plant growth and development. Effects of pesticides on plants have not been measured in both susceptible and tolerant plant species at cellular and molecular levels, therefore we have little or no biochemical information as to why susceptible and resistant plant species exist.

Basically a herbicide is a chemical which may be used to kill plant species growing in places where they are not wanted. Therefore most herbicides are phytotoxic substances. In view of the importance of pesticides for quality and quantity of food and fiber productions and for safety of use, it is apparent that a better understanding of modes of action of pesticides in both susceptible and tolerant plants is needed. Such an understanding could lead to a rapid and reliable screening procedure for substitute herbicides and plant growth regulators.

With this background, I shall review with you our project #68-01-2482, which has for its study objective the rapid identification and measurement of biochemical changes in plant cells due to exposure to herbicides. The Department of Botany, University of Michigan, was awarded this study contract. Three professors, three technicians, and five graduate assistants have been engaged since July 1974 on this project. We in the Criteria and Evaluation Division of the Office of Pesticide Programs have set the following criteria for the rapid screen procedure.

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*Criteria and Evaluation Division, Office of Pesticide Programs, EPA

The scope of work is to positively identify three or more modes of action simultaneously, including inhibition of mitochondrial respiration or electron transport, inhibition of protein synthesis controlled by DNA-dependent RNA, and inhibition of photosynthesis and Hill reaction. The acceptable biochemical method must be rapid, reliable, and accommodate the following: 1) detect possible simultaneous presence of the above modes of action in a single susceptible plant species; 2) provide reliable data within 48 hours; 3) be reliable at the 95 percent level; 4) allow multiple, simultaneous analysis.

DR. SIKKA: Dr. Hayashi, what other plant species could be used?

DR. HAYASHI: So far, we are using one plant species, soybeans.

Table 1 shows common names of herbicides which are used in this research project. As you see, this is a chemical group classification of herbicides: phyenoxys, phenol, arsenical, bipyridylium, carbamates, thiocarbamate, dinitroaniline, triazines, triazole, ureas, and uracil.

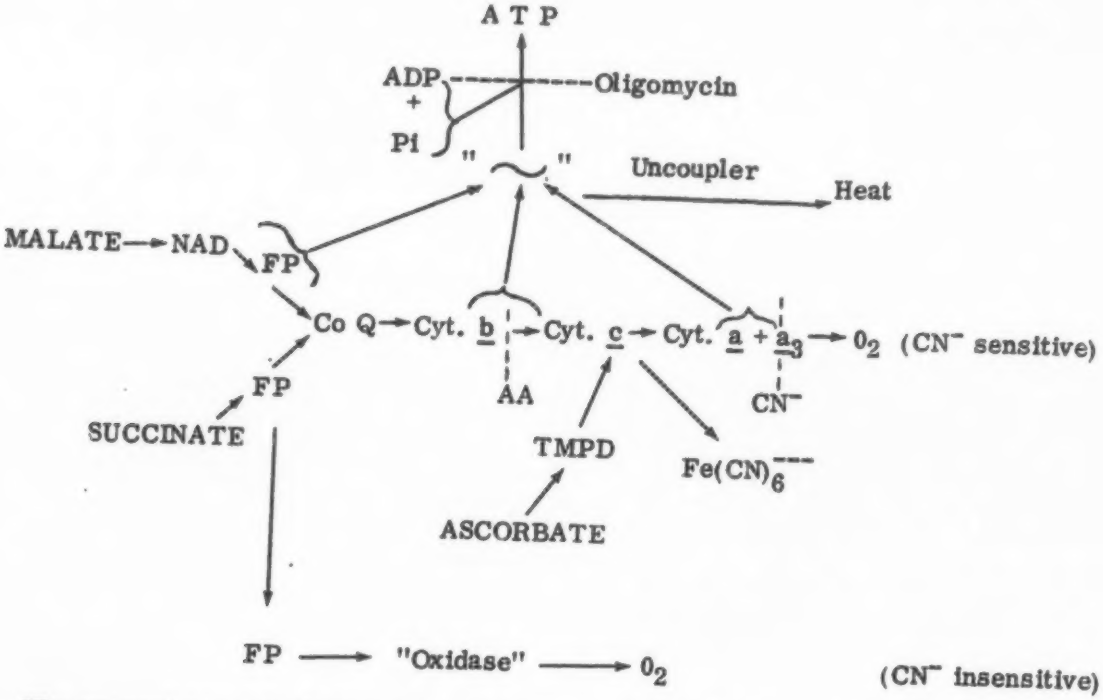
Figure 1 shows a schematic presentation of electron transfer and energy transfer systems in plant mitochondria. Plant mitochondria are believed to contain two electron transport chains. The main phosphorylative chain is inhibited by cyanide terminally and by antimycin A (abbreviated AA in this figure) in the middle, and a non-phosphorylation chain withdraws electrons from the flavo protein (abbreviated as FP in the figure) level to oxygen via a flavo protein and a second oxidase. The second chain is cyanide- and antimycin A-resistant. The exact nature of the cyanide-resistant chain has not been clarified yet, but it is estimated to consist of about 25 percent of total respiratory activity of mung

Table 1: Herbicides Studied

Chemical Group	Common Name	Chemical Name	Source
Phenoxys	2, 4-D 2, 4, 5-T	(2, 4-dichlorophenoxy)acetic acid (2, 4, 5-trichlorophenoxy)acetic acid	Sigma EPA
Arsenicals	DSMA Cacodylate	Disodium methylarsonate Hydroxydimethylarsine oxide	Ansul Baker
Bipyridylium	Diquat	6, 7-dihydrodipyrido (1, 2-a:2', 1'-c) pyrazinedium ion	Ortho
Carbamates	Propham Chlorpropham	Isopropylcarbanilate Isopropyl-m-chlorocarbanilate	K & K K & K
Thiocarbamate	Diallate	s-(2, 3-dichloroallyl)-diisopropylthiocarbamate	Monsanto
Dinitroaniline	Trifluralin	α, α, α-trifluoro-2, 4-dinitro-N, N-dipropyl-p-toluidine	Lilly
Phenol	DNOC	4, 6-dinitro-o-cresol	Baker
Triazines	Atrazine Simazine	2-Chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine 2-Chloro-4, 6-bis(ethylamino)-s-triazine	Ciba-Geigy Ciba-Geigy
Triazole	Amitrole	3-amino-1, 2, 4-triazole	Aldrich
Ureas	Monuron Diuron (DCMU) Karbutilate	3-(p-Chlorophenyl)-1, 1-dimethylurea 3-(3, 4-Dichlorophenyl)-1, 1-dimethylurea tert-butylcarbamic acid ester with 3-(m-hydroxyphenyl)-1, 1-dimethylurea	Aldrich K & K FMC
Uracil	Bromacil	5-Bromo-3-sec-butyl-6-methyluracil	DuPont

bean mitochondria. The main chain is coupled to the energy conservation pathway of ATP formation at the three sites. Although the exact mechanism by which ATP is formed is not elucidated yet, it is believed to go through a so-called "high-energy" intermediate state.

Figure 1



A T P
ADP
+
Pi
Oligomycin
" ~ "
Uncoupler
Heat
MALATE → NAD
FP
Co Q → Cyt. b → Cyt. c → Cyt. a + a3 → O2 (CN⁻ sensitive)
AA
CN⁻
FP
SUCCINATE
TMPD
Fe(CN)6---
ASCORBATE
FP → "Oxidase" → O2
(CN⁻ insensitive)

The current assay techniques for studying possible inhibitions by herbicides or mitochondrial activity depend heavily on measurement of oxygen consumption. Oxygen uptake is started by an addition of a substrate, and it is further stimulated by addition of ADP to the reaction medium which contains inorganic phosphate.

Tables 2 and 3 show the results of 15 herbicides tested on respiratory activity. Seven herbicides gave no effect at the starting minimal concentrations on mitochondrial activity. Bromacil, chlorpropham, diuron, DNOC, and propham only showed a slight effect at mM concentrations two orders of magnitude less.

Table 2: Effects of Weakly Active Herbicides
on Mitochondrial Activity*

Herbicide	Substrate	Ki (mM)	Remarks
Bromacil	Succinate	---	Max. inhibition 37% at 10 mM
	Malate	1.6	80% inhbition at 10 mM
Chlorpropham	Succinate	ca. 5	Max. inhibition 40% at 10 mM
	Malate	0.7	80% inhibition at 10 mM
Propham	Succinate	---	Max. inhibition 40% at 10 mM
	Malate	0.8	85% inhibition at 10 mM
Diuron	Succinate	---	Slight uncoupling at 0.3-1 mM
	Malate	0.2	90% inhibition at 3 mM
Monuron	Succinate	---	Max. uncoupling at 0.3-1 mM
	Malate	---	Uncoupling at 1 mM
2,4-D	Succinate	ca. 0.7	Max. inhibition 50% at 3 mM
	Malate	ca. 0.6	Max. inhibition 75% at 3 mM
2,4,5-T	Succinate	0.9	Max. inhibition 95% at 3 mM
	Malate	0.2	Max. inhibition 95% at 3 mM

* Effects of weakly active herbicides on mitochondrial activity. Various concentrations of a herbicide are given at the second state 4, and the effects on that state or on the subsequent state 3 rate (induced with about 150 µM ADP) were examined. Substrate concentration used: 10 mM succinate or 30 mM malate. The Ki value refers to the concentration of a herbicide which inhibits 50 percent of the second state 3 rate.

Table 3: Effects of Herbicides on Respiratory Activity of Isolated Corn Root Tips*

Herbicide	Concentration Used (M)	Effect on	
		O_2 Uptake	CO_2 Evolution
Amitrole	10^{-3}	0	0
	10^{-4}	0	0
Atrazine	10^{-3}	0	0
	10^{-4}	0	0
Bromacil	10^{-3}	0	0
	10^{-4}	0	0
Cacodylate	10^{-3}	0	157% up
	10^{-4}	0	59% up
Chlorpropham	10^{-3}	0	0
	10^{-4}	0	0
2,4-D	10^{-3}	0	85% up
Diallate	10^{-3}	0	0
	10^{-4}	0	0
Diquat	10^{-3}	0	0
Diuron	10^{-3}	0	0
DNOC	10^{-3}	55% down	21% down
	10^{-4}	45% up	203% up
	10^{-5}	0	0
DSMA	10^{-3}	0	0
Karbutilate	10^{-3}	0	0
Monuron	10^{-3}	0	0
Propham	10^{-3}	0	0
Simazine	10^{-3}	0	0
2,4,5-T	10^{-3}	0	75% up
Trifluralin	10^{-3}	0	0

*Effects of herbicides on respiratory activity of isolated corn root tips. 0 denotes no significant effect of herbicide; up signifies percent increase above control; down means percent decrease. Control values are 570 nmoles/min.•g fresh weight for oxygen uptake and 605 nmoles/min.•g fresh weight for carbon dioxide evolution.

Table 4: Effects of Herbicides on Mitochondrial Activities, Using an Oxygen Electrode

Herbicide	Concentrations (mM) Tested Which Showed				Substrate Used
	No Effect	Uncoupling	Electron Transfer Inhibition	Energy Transfer Inhibition	
Amitrole	3.0	---	---	---	Succinate and Malate
Atrazine	3.0	---	---	---	Succinate and Malate
Bromacil	---	---	1 - 5	---	Succinate and Malate
Cacodylate	10.0	---	---	---	Succinate and Malate
Chlorpropham	---	10.0	---	---	Succinate
	---	---	3 - 10	---	Malate
Diallate	---	---	> 0.05*	---	Succinate and Malate
Diquot	3.0	---	---	---	Succinate and Malate
Diuron	---	---	1 - 3	---	Succinate and Malate
DNOC	---	0.001 - 1	>1	---	Succinate
DSMA	3.0	---	---	---	Succinate and Malate
Karbutilate	3.0	---	---	---	Succinate and Malate
Monuron	---	3.0	---	---	Succinate and Malate
Propham	---	> 3.0	1 - 10	---	Succinate and Malate
Simazine	3.0	---	---	---	Succinate and Malate
Trifluralin	---	> 0.02	> 0.01	---	Succinate and Malate

*Diallate titration curves are different for succinate oxidation and malate oxidation.

As exemplified by DNOC which acted to uncouple at low concentrations and caused incomplete inhibition of electron transport at high concentrations, the herbicides which affect mitochondrial activity at the mM level may affect other metabolic activities more potently. It is of interest to note that the inhibitors of the Hill reaction, such as atrazine and simazine, do not affect mitochondrial activity at a mM concentration, while monuron, an inhibitor of photosystem II, induces particle uncoupling at a high concentration. Another frequently used inhibitor of photosystem II, diuron, inhibits respiratory electron transfer at the high concentration.

These results, together with insensitivity of the chloroplast system to antimycin A, oligomycin and DNP, can be considered to indicate that the nature of electron transport and coupled phosphorylation is different in mitochondria and chloroplast.

Of the 15 herbicides tested, three gave no effect in the oxygen evaluation assay at a mM concentration range. All the rest inhibited electron transfer at concentrations less than one mM. Most potent inhibitors of electron transfer are bromacil, followed by atrazine, karbutilate, monuron, simazine, and trifluralin. Chlorpropham and propham are relatively ineffective, requiring concentrations one order of magnitude higher than the above group.

The results from the herbicidal effects on the oxygen consumption assay show that most herbicides do not affect the photosystem I activity at mM concentrations. It can be concluded that most herbicides inhibit the photosystem II activity.

Of the 15 herbicides tested, four herbicides have an inhibitory effect on DNA synthesis and five herbicides inhibited protein synthesis when we tested using ^{14}C-leucine plus ^{3}H-thymidine or ^{14}C-uridine incorporated into DNA and RNA fractions of corn root tip cells.

Table 5: Effect of Five Herbicides on Incorporation of Precursors into DNA, RNA, and Protein Fractions of Corn Root Tips*

Herbicide	Conc. Used (M)	DNA		RNA		Protein	
		CPM	% of Control	CPM	% of Control	CPM	% of Control
Simazine	0	3,280	100	2,040	100	16,000	100
	10^{-5}	3,210	98	2,210	109	17,550	110
	10^{-4}	3,080	94	1,700	83	17,500	110
Monuron	0	4,630	100	2,450	100	25,200	100
	10^{-5}	5,530	120	1,300	53	31,700	126
	10^{-4}	5,210	113	1,150	47	30,000	120
	10^{-3}	2,780	60	650	27	27,200	108
Karbutilate	0	4,300	100	1,970	100	21,500	100
	10^{-5}	4,300	100	2,310	118	25,100	117
	10^{-4}	4,660	108	1,820	92	28,500	133
	10^{-3}	3,260	76	1,300	66	24,200	113
Chlorpropham	0	4,230	100	1,830	100	23,100	100
	10^{-5}	4,170	99	1,830	100	23,500	102
	10^{-4}	3,880	92	1,840	101	20,700	94
Bromacil	0	6,380	100	1,860	100	24,900	100
	10^{-5}	6,360	100	2,570	138	29,500	118
	10^{-4}	4,430	69	2,160	115	24,000	97
	10^{-3}	1,368	22	630	34	15,800	63

*Twenty-five root tips were incubated with ^{14}C-leucine + ^{3}H-thymidine or ^{14}C-uridine for 2 hours and fractionated into DNA, RNA, and protein with the enzymatic method described in the last quarterly report. Incorporation in CPM is an average of duplicate runs expressed on the basis of 25 root tips.

Table 6: Effect of Some Herbicides on Incorporation of Precursors into DNA, RNA, and Protein Fractions of Corn Root Tip Cells*

Herbicide	Concentration Used (M)	DNA		RNA		Protein	
		CPM	% of Control	CPM	% of Control	CPM	% of Control
Atrazine	0	9,780	100	1,484	100	63,520	100
	10^{-5}	9,240	94	1,135	76	59,610	94
	10^{-4}	9,080	93	692	47	52,140	82
	10^{-3}	980	10	163	11	10,940	17.
Diallate	0	9,860	100	2,175	100	83,400	100
	2×10^{-5}	10,710	104	2,250	103	123,610	148
	10^{-4}	7,790	79	1,720	68	42,540	51
DNOC	0	12,900	100	790	100	104,600	100
	2×10^{-5}	5,180	40	540	68	18,730	18
	10^{-4}	790	6	115	15	2,080	2
	2×10^{-4}	60	0.5	160	20	200	0.2
Trifluralin	0	3,070	100	740	100	18,410	100
	2×10^{-6}	3,535	115	730	99	16,970	92
	2×10^{-5}	3,340	109	810	110	16,030	87
	10^{-4}	2,740	89	830	112	11,820	64

*Ten root tips were incubated with ^{14}C-leucine + ^{3}H-thymidine or ^{14}C-uridine for 2 hours and fractionated into DNA, RNA, and protein as described in the text. Incorporation in CPM is an average of duplicate runs expressed on the basis of 10 root tips.

We also examined morphological changes of plant material with or without herbicide application. An electron microscopic study is important for evaluating physiological and chemical modes of action.

Figures 2 and 3 show some results of our morphological experimental work. When we treat with 10 micro trifluralin, the number of microtubules along the cell wall was reduced. Figure 2 is a water control for the spindle fiber.

Figure 2

Untreated Cell Wall (Water Control) 17,000 X

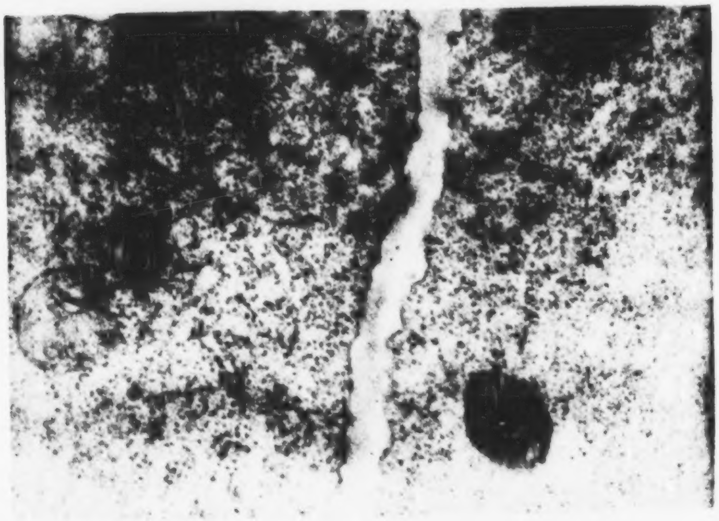


Figure 3

Treated with Trifluralin 10 mM, 17,000 X

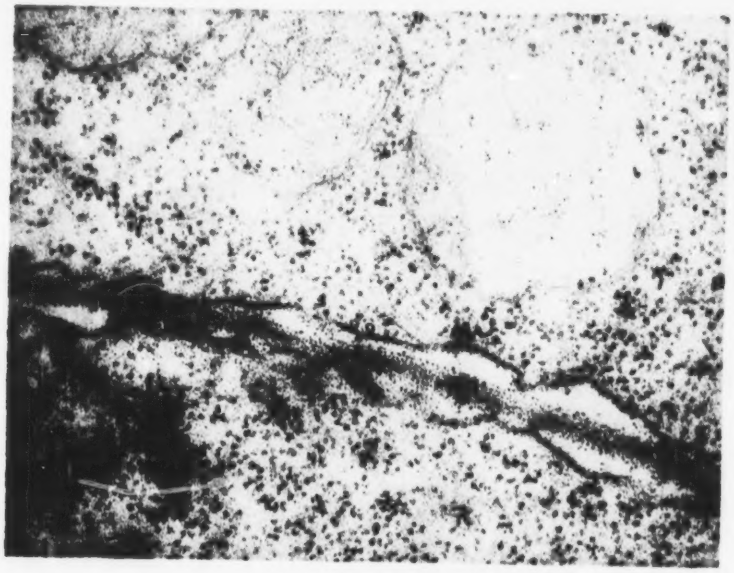
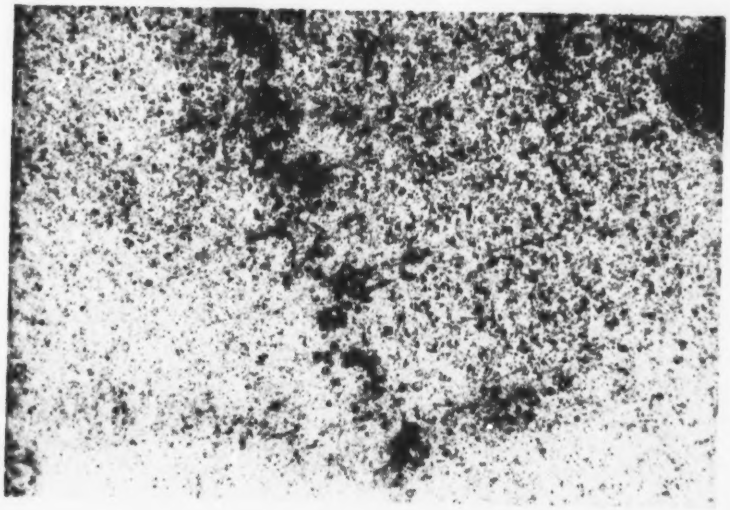


Figure 4

Untreated Spindle Fiber (Water Control) 17,000 X



After treatment with trifluralin the spindle fibers disappeared completely (See Figures 4 and 5). Figure 6 shows a water control of bromacil. When we apply 10^{-6}M of bromacil, you can see the enlarged mitochondria and the donut shaped mitochondria (Figure 7). With 10^{-6}M treatment we sometimes cannot see the biochemical response, but we may be able to detect some mitochondrial or morphological changes using an electron microscope.

Figure 5

Treated with Trifluralin 10 mM, 17,000 X

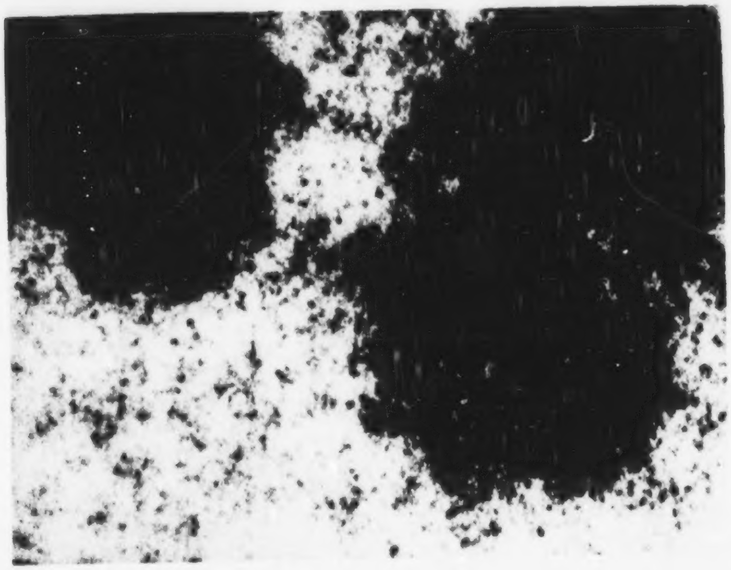


Figure 6

Untreated Mitochondria (Water Control) 25,000 X

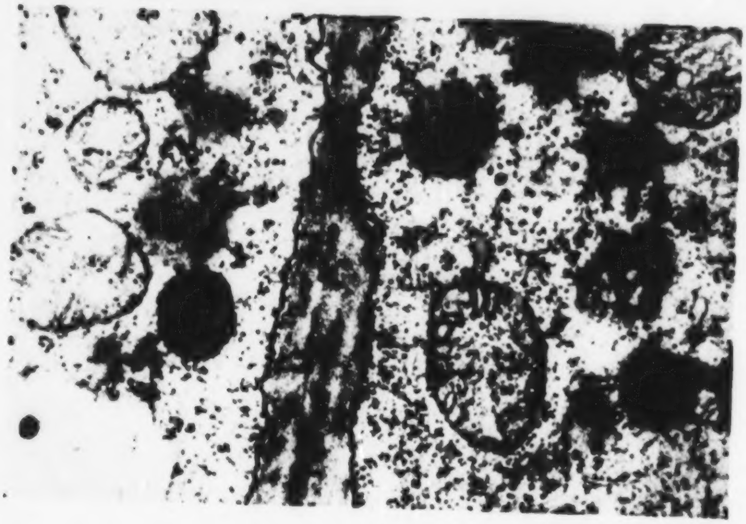
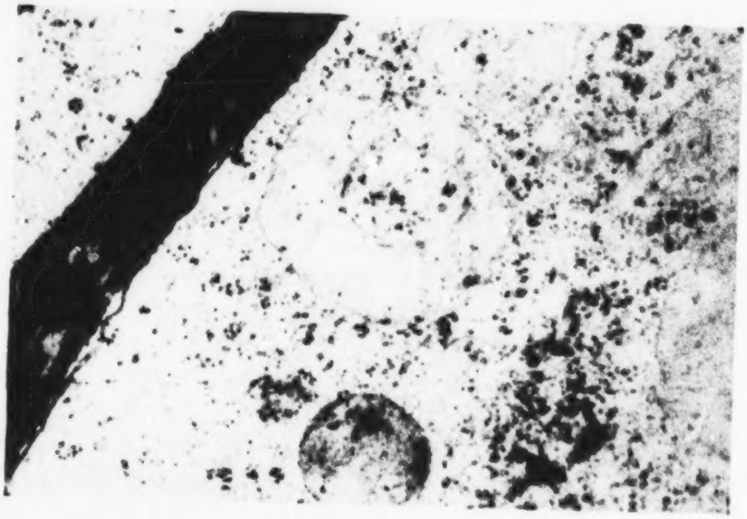


Figure 7

Treated with Bromacil 10^{-6}M, 25,000 X



It is possible to evaluate the mode of action of the herbicide by the morphological changes. From the various previously described experimental work, we concluded that the following screening methods will be reasonable and reliable for phytotoxicity.

Once more I'd like to explain the flow of electrons from water to carbon dioxide in plant cells. Figure 8 is a schematic chart of how to metabolize from water molecule to carbohydrate, or some other carbon compounds with carbon dioxide. This is also called the Hill reaction. And as far as we studied, many herbicides are effective to photosystem II. We can evaluate mode of action for those herbicides by the changes of electron transport systems.

Our proposed evaluation method of phytotoxicity has four steps. For post-emergence herbicides we put the whole intact plant into the chamber. We then apply the herbicide either on the top or the root. We'll measure carbon dioxide using the IR gas analyzer. The root growth inhibition will be measured by IR gas analyzers and oxygen electron. In the case of pre-emergence herbicides, we put the seed on a petri dish, keeping it in the chamber, and measuring carbon dioxide evolution using an IR gas analyzer (Figures 9 and 10).

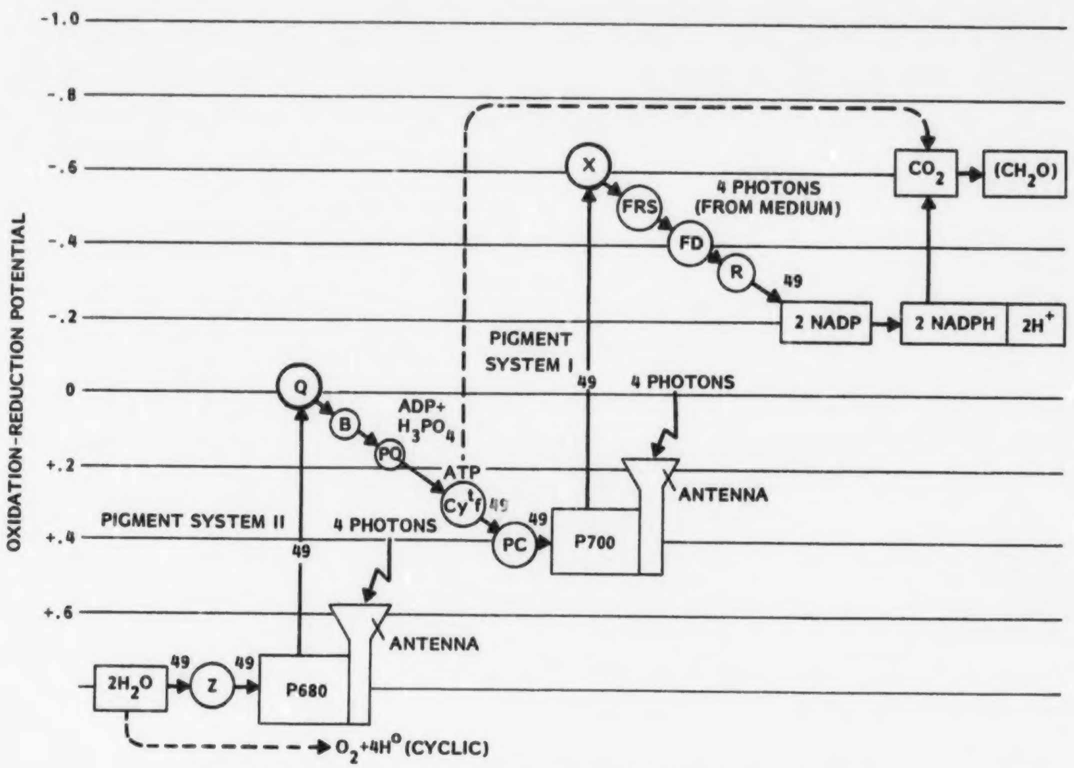
I'll discuss the second step later. The third step is to measure respiratory changes in mitochondrial suspension solution with or without herbicide application. Step four is to check for morphological changes using the electron microscope.

How we are currently constructing the instrument is diagrammed in Figure 11. This is the second step. Our preliminary experiments in which a leaf was held between two color filters indicate that the changes of leaf fluorescence can be very easily detected visually. The construction of the box which houses a leaf sample and two color filters must be completed before obtaining qualitatively comparative data for screening various herbicides.

The instrument for the instant evaluation method has two filters. The primary filter has 450 mM absorption, and the secondary filter is 600 mM. We put a sample leaf between the two filters and measure fluorescence intensity.

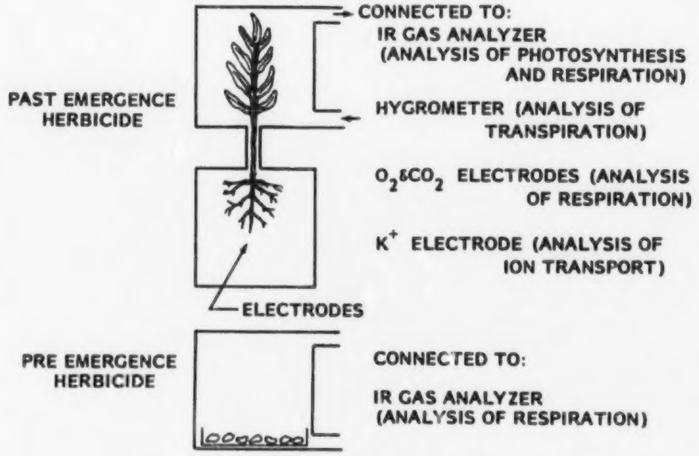
Figure 8

Electron Flow System in Chloroplast


-1.0
-.8
-.6
-.4
-.2
0
+.2
+.4
+.6
OXIDATION-REDUCTION POTENTIAL
X
FRS
FD
R
49
4 PHOTONS
(FROM MEDIUM)
CO_2
(CH_2O)
2 NADP
2 NADPH
$2H^+$
PIGMENT
SYSTEM I
49
4 PHOTONS
Q
B
PQ
ADP+
H_3PO_4
ATP
Cy^tf
49
49
PC
P700
ANTENNA
PIGMENT SYSTEM II
4 PHOTONS
49
ANTENNA
$2H_2O$
49
Z
49
P680
O_2+4H^0 (CYCLIC)

FLOW OF ELECTRONS FROM WATER TO CARBON DIOXIDE IN PLANT CELLS

Figure 9


CONNECTED TO:
IR GAS ANALYZER
(ANALYSIS OF PHOTOSYNTHESIS
AND RESPIRATION)
PAST EMERGENCE
HERBICIDE
HYGROMETER (ANALYSIS OF
TRANSPIRATION)
O_2&CO_2 ELECTRODES (ANALYSIS
OF RESPIRATION)
K^+ ELECTRODE (ANALYSIS OF
ION TRANSPORT)
ELECTRODES
PRE EMERGENCE
HERBICIDE
CONNECTED TO:
IR GAS ANALYZER
(ANALYSIS OF RESPIRATION)

PROPOSED EVALUATION METHOD FOR PHYTOTOXICITY-1

STEP-I

Figure 10

CHLOROPHYLL FLUORESCENCE DETECTION

STEP-II

MITOCHONDRIAL RESPIRATION DETECTION

STEP-III

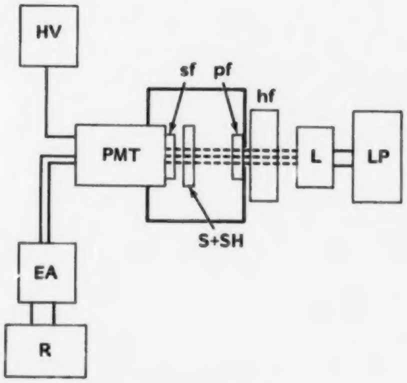
ANALYSIS OF ULTRASTRUCTURAL CHANGES

STEP-IV

PROPOSED EVALUATION METHOD FOR PHYTOTOXICITY-2

Figure 11

SCHEMATIC PRESENTATION OF THE INSTRUMENT TO MEASURE LEAF FLUORESCENCE



LP --LIGHT POWER SUPPLY
L --LAMP
hf --HEAT FILTER
pf --PRIMARY FILTER (CA. 450mu)
sf --SECONDARY FILTER (CA. 600mu)
S+SH --SAMPLE (LEAF) IN A SAMPLE HOLDER
PMT --RED SENSITIVE PHOTOMULTIPLIER TUBE
HV --1.5 KV POWER SUPPLY FOR PHOTOMULTIPLIER TUBE
EA --ELECTROMETER AMPLIFIER
R --RECORDER

I hope someday the basic concept of our screening technique using the electron transfer system or respiratory system can be used in establishment of an evaluation method for all biologically active chemicals, including pesticides. Toxicity to mammalian and plant cells would be evaluated by changes of electron transport at the molecular level using some unit of evaluation instead of LD_{50} as at present. I believe that the electron transfer system will be a useful screening technique for evaluation of substitute chemicals among the pesticides.

PROGRAM OF THE OFFICE OF TOXIC SUBSTANCES IN RELATION TO MICROCOSM METHODOLOGY DEVELOPMENT, AND ECOLOGICAL EFFECTS PROGRAM OF THE OFFICE OF TOXIC SUBSTANCES

Carter Schuth*

I'd like to thank the Office of Research and Development and the Office of Pesticide Programs for this opportunity to advertise the environmental fate and effects testing program of the Office of Toxic Substances. As you may know, the several versions of the Toxic Substances Control Act currently pending in the Congress all have provisions for EPA to require certain types of test data from industry on chemicals and chemical classes in order to determine if a chemical substance presents an unreasonable risk to health or the environment. The task for us is thus not the testing of chemicals but the description and interpretation of adequate test methodologies, the results of which will serve as bases for decision-making as to the need for regulatory action by EPA. I would like to discuss with you this morning the part of our program which is directed toward environmental fate and effects testing, the objective of which is the "evaluation and demonstration of methods for environmental testing of chemicals."

As an aside, let me elaborate a bit on the significance of environmental fate and/or effects. For reasons which are probably best described as bureaucratic or for the sake of simplicity and organization, we tend to deal with fate separately from effects, especially in the discussion which is to follow. More emphasis seems to be placed on fate in our program at this time. This is due to our feeling that an understanding of environmental fate is essential in arriving at some estimate of the probability of observing (and causing) environmental and human health effects, i.e., exposure. A need crying to be filled is the development and demonstration of reliable and simple screening techniques for these effects. Bear in mind that Bob Metcalf's original idea was to develop in his model ecosystem a "white rat" for the environmental toxicologist as an appropriate laboratory model. It is not sufficient to estimate fate without some attention to possible effects. I would strongly encourage the behavioral work of the Gulf Breeze Lab and others as a promising enterprise. In addition, I'd like to pass on a comment brought to my attention by John Buckley. We can all collect reams of data from any given model, but as regulators and the regulated, what is the information we need and, very importantly, what is the information we don't need?

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*Office of Toxic Substances, EPA

Now, the return to my topic -- I wish to discuss with you the environmental testing activities of the Office of Toxic Substances. In support of this objective is a contract effort involving three separate but related activities--methodology assessments; demonstration projects; and chemical- or problem-specific information services. Whereas I want to concentrate my comments on the second topic, I will briefly mention the other two.

A number of methodology assessments have been completed or are in the final stages of completion. These are state-of-the-art reviews involving a comprehensive literature search and review, interviews involving a comprehensive literature search and review, interviews with specialists in the various fields, and evaluations by the contractor as to the usefulness and convenience of the test methodologies. The following topics comprise a listing of the reports that have been undertaken by this program:

- Survey of industrial test practices
- Persistence and routes of degradation of chemicals in the environment
- Transport of chemicals in the environment
- Methods to assess the effects of chemicals on terrestrial animal species
- Methods to assess the effects of chemicals on plants

Most have been completed and are available through the National Technical Information Service in Springfield, Virginia. I have prepared a handout with the exact title, an abstract, and information on ordering the reports. If there are insufficient copies of this for those who are interested, I will be glad to mail this information to anyone who gives me his or her name and mailing address at the end of this session.

In addition to information on the methodologies for determining the environmental fate of chemicals, we are sometimes interested in knowing what information is available on the fate or effects of specific chemicals. We are also interested in keeping abreast of current developments in the various methodologies. For these services, we turn to task order contracts which can produce information and reports on a very short schedule.

Several reports under the category of chemical-specific information services are:

- Environmental fate of selected carcinogens
- Benzene: environmental sources of contamination, ambient levels, and fate
- Environmental fate of selected PNAH's
- Environmental fate of selected aza-arenes

This information is gathered from the published literature, scientists working the field, and educated guesses on the part of the contractors. To date we have investigated the environmental fate of six carcinogens (as defined by the Occupational Health and Safety Administration) and benzene. Ongoing are studies of the environmental fate of polynuclear aromatic hydrogens including some nitrogen heterocycles.

I'd like to turn now to the part of our program that involves model systems directly. In the following list of demonstration projects, the studies listed under parts 1 and 2 have been started under FY-75 funding.

1. Reproducibility and variability
   Modular food chain
   Model ecosystem

2. Validation studies
   Model stream

3. Physical-chemical measurements
   Octanol/water partitioning coefficients
   Water solubility
   Vapor pressure

The third category is proposed for this year.

Among the many criticisms of laboratory models for determining environmental fate and/or effects is the recurring charge that we know very little or nothing about the reproducibility and variability of the various systems. In response to this, we have undertaken the two studies shown in part 1. Dr. Thomas Johnson of the Fish Pesticide Laboratory in Columbia, Missouri has been working for several years on what he has called a "modular food chain." This model system appeals to us as being relatively uncomplicated, inexpensive, and indicative of actual environmental conditions. I need not mention that concentration of toxicants in food chains is of primary concern to EPA. In order to assess the modular food chain's utility in a regulatory mode, we have signed an interagency agreement with the Department of the Interior for the Fish Pesticide Laboratory to investigate the reproducibility and variability of the modular food chain using four chemicals and various concentrations of these chemicals. By analogy, the model terrestrial-aquatic ecosystem first described by Metcalf et al. has been met with less than universal acceptance. We have signed a contract with the Illinois Natural History Survey to undertake a study of the reproducibility of that model using the same four compounds. In this way, we hope to get good comparisons of each system.

The intent of this project is to better understand the two systems rather than generate new information about potentially hazardous chemicals. In fact, it can be viewed as the study of two simple and inexpensive systems using "benchmark" chemicals. In addition to studying the reproducibility of each system with respect to the same chemicals, we are interested in assessing the utility of each system with respect to certain types of chemicals. So, we have set up criteria for the selection of chemicals in order to achieve this comparison. We wish to look at compounds which display a range of C-14 isotopes as well as being well-characterized as to possible metabolites. We have selected decachlorobiphenyl, hexachlorobenzene, di-2-ethylhexyl phthalate, and atrazine.

In addition to wanting information on the reliability of the simpler models, we ask ourselves what can they tell us about the "real world." Dr. Howard Johnson of the Michigan State University has signed a contract to study the fate and effects of two of the previously mentioned chemicals in model stream communities. These model streams have been set up in a coverted fish hatchery in Paris, Michigan. They are supplied with communities of organisms and pristine water from a stream which has been diverted to flow through the concrete channels in the hatchery. Dr. Johnson and his staff will determine the fate of the chemicals in the various trophic levels of the model streams as well as observe effects of the chemicals on the organisms. Our rationale in selecting the model streams involved the belief that field studies are both very difficult and expensive. Analogies of the model stream results and monitoring data will be possible and will be useful in validation of our models and the environment we strive to protect.

It is our intention as regulators to require of industry only the necessary information on its products that is needed for responsible decision-making. It has been suggested to us that these model systems are too complicated and that the measurement of simple physical and chemical parameters gives the same information. This is an area that we are now investigating and feel sure will fit into the program that I've described to you this morning.

The last category encompasses the utility of physical and chemical parameters to indicate the environmental fate and transport of chemicals. As we discussed yesterday, these numbers are useful in the utilization of models being developed as well as for comparisons with "known" compounds.

ANALYTICAL METHODS DEVELOPMENT

Dr. James Hill*

NOT AVAILABLE

FOR

PUBLICATION

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*Environmental Research Laboratory, Athens, Georgia

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SPECIAL INTEREST TOPICS AND GENERAL DISCUSSION

DR. BUCKLEY: We may have a fairly lively discussion over the next hour or two, and I don't really have any ground rules as to how vigorous or rough or contrary wise we ought to be. I have a number of observations to make, and some of them are perhaps designed to arouse a certain amount of controversy. Some of them are intended to see if we can figure out some ways in which we can do business a little bit better.

Some of them may be controversial in a sense because they rather run counter to the traditional ways in which science is done, of trying to understand all about everything, and I'd like to turn some of that around. It's true that I'm in the research part of EPA and not in the regulatory part, but I am constantly and continuously made aware of the fact that my only reason for existence is to serve that part of the Agency which is concerned with regulation. So it seems to me there are some general questions that we need to think about.

Carter Schuth mentioned this morning that we need to somehow or another concentrate on what it is that we need to know. To turn that the other way around, what are the things that we can get along without knowing? At the San Antonio conference on evaluation of chemicals in the environment -- I don't have a copy with me, and my quote may not be precise -- I pointed out, and I would emphasize again now, that the game we're involved in really is trying to acquire the least amount of information necessary as a basis for decisions. It's not how can we find out as much as possible about a particular substance or what happens. I realize that not all of us have the same crystal balls, and that if we do look in the crystal ball, we may not all see the same image. I think we really ought to be thinking as deeply as we can about what things about chemicals and their interactions with organisms are the important ones that are necessary so that sensible judgments can be made.

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There's kind of an irreverent question that I keep asking people when they propose a different kind of research. It is, "What can I do with that piece of information if you generate it that I can't do without it?" or "What can I do better if you tell me that?" And then sometimes after they come with a piece of information which has been painstakingly gathered, it seems to me very reasonable to ask, "So what?" I don't mean it in a flippant sense. But I mean, if you tell me that, say, the nitrogen cycle is interfered with in a particular circumstance, that doesn't help me much if I'm an administrator. What I really want to know is, "What difference does it make in terms of human well-being?" And it's just possible it doesn't make a bit of difference in human terms. We know that some of the fungicides that are used do interfere with the nitrification process, and so long as we're using these on agricultural lands where we apply fertilizers with a liberal hand, a couple of odd pounds of nitrogen plus or minus in the cost of production don't make that much difference.

On the other hand, if you do this in a forest system, where you don't add nitrogen, maybe the answer to the question is very different. So I think there are some things to be thought about in different terms. Not all ecosystems are equally important in terms of man. Not all ecosystems are equally vulnerable. Within the elements of vulnerability, not all processes are equally vulnerable in all systems.

Somehow or another you have to think about these things in terms of finally deciding whether you're going to allow a particular chemical to be used in a particular way. Back of all this hangs the question of "What happens to the chemical in the environment?" Now we've got some real problems. We don't know what's happened to a lot of chemicals we've already put in the environment. And now we're talking under the Toxic Substances Act of determining what would happen to a chemical if we did put it in the environment. I submit that that's worthy of a considerable amount of thought.

After we have our minds all made up about where it would go and how long it would be there, then we're faced with a question of "What does it do while it's there?"

Finally, I suppose, the payoff question is "What difference does it make if it does cause some particular effect?" Those are not scientifically phrased questions, but basically they are what we're about here. What we are trying to do here, I think, is to understand which things are important to learn about, how little we can get by with in our knowledge of these, and to give some reasonable projection of what things will be like.

I guess that's the end of my lecture as such, but I had two or three other things that came to mind as I listened. I think when Carter Schuth was talking this morning about not being interested just in fate and transport, I'm not interested solely in that either. I think the body of information we have in general and our ability to make some kinds of estimates on effects is so much greater than our ability to make reasonable estimates on what happens to the chemical, that I'm anxious at the moment to push studies of fate and transport. It's not because I think the other things are unimportant, but I have some gut feeling that I can cope with that part of it better.

Another thing, I didn't hear much comment after Pete Schoor's presentation yesterday when he was talking about two elements of behavior that were being looked at, and I guess I think behavior is a subtle sort of thing to get hold of, and I think it's something that's been grossly underdone. I think that's true in terms of what happens in the environment. I know it's true in terms of what happens to people. And this is a general purpose comment, as far as behavioral research, which I expect to make to the toxicologists worried about human well-being as well as here.

I think there are some very sensitive indicators that may be applicable to the "real world" that maybe we ought to be exploiting somewhat more. I hope that those of you who are working actively with these model systems and systems models will put your heads together on how we can get at the important things, what the important things are, where there are some promising leads that haven't been looked at, and how far we can extrapolate from what we get.

In these terms, what is it that these systems represent? When we feed a mouse a compound to see whether a tumor is produced, it isn't really that we're worried about the mouse population. What we're really worried about is man. We've made a judgment that says mice and rats and some other animals in some sense or another represent man, and we do extrapolate. We worry about how good the extrapolations are.

The problem I see here is that we really don't worry enough about what our tests are meant to represent and how far we can legitimately extrapolate from them and with what levels of confidence. I hope in your discussions that you'll think about that. I think the work of standardization or comparability that Carter Schuth was talking about this morning is important. If Joe Smith does it, and Harry Jones, can they both start from sort of the same recipe on how you do it? Do they come out the same way or are their results quite different?

I don't think we've done enough of such comparisons. I think this undertaking to look at comparability among systems that we're inclined to use is probably very useful. To what degree do they represent the real world or portions of the real world? The final thing that I would keep stressing is to think about what questions are important.

I would hope you can start at the most integrated level of the system that you can afford to deal with and undertake to see whether this net end result shows anything. If it doesn't, for the moment at least, I would be inclined to turn to something else and go on. Now I may have to come back to this later on, but as a first cut, it seems to me we ought to do that.

That's not only what I meant to say, but it's probably more than I meant to say.

DR. EATON: I'm curious to know what the other behavioral responses you referred to were.

DR. BUCKLEY: Well, I guess I was thinking in Pete Schoor's talk, what he was using in that particular case was predation and ability to escape predation as a sensitive indicator of effect on the prey. Provided you don't end up with levels that are harmful to the predator, more easily available prey may be a good thing on a temporary basis for the predator. Or you can turn it the other way around, and the inability to escape the predator may in fact be distinctly harmful to the prey species.

Obviously you have to examine it from both ends in terms of the net biological effect. What I was driving at was that if you go back to what we used to do in looking at an effect of a chemical on the estuarine environment, we tried static tests in jars, and we discovered that didn't work very well. So then we decided we had to have flow through systems. So we did that. Then we looked at shrimp -- pink shrimp, brown shrimp, white shrimp, even grass shrimp, but mostly not grass shrimp, the others -- to come up with some 96-hour LC_{50}s. We discovered that shrimp were difficult to work with that way, so we threw out the LC_{50}, and we began talking about an EC_{50}, which was an effect concentration, and the reason we did that was because the shrimp would become immobilized, but it just wouldn't die within 96 hours.

And, you know, it was reasonable to assume that, were it out on the open ocean and immobile, it probably wouldn't survive very long. Then you begin to wonder if there weren't levels lower than this, where ability to move would be impaired, but not in a way that you could observe, and I think what people at Gulf Breeze did was to put two species together and let the predator be the indicator of the effect on the prey species, a behavioral characteristic in its inability to escape, to perform in its usual way.

I suspect there may be many clues of this sort, if we look at what we've been doing, that we haven't yet exploited. It seems to me almost impossible that this is the sole example of that kind of thing. Does that answer, John?

DR. EATON: I was wondering if you had more examples.

DR. BUCKLEY: Well, yes. There is another one. In work on the lugworm, and again it's behavior in that sense, it's looking at the configuration of the surface and whether treated and control worms make the same patterns. It is a lot different from an LC_{50} or an LD_{50} -- perhaps more sensitive -- I don't know enough about the lugworm to answer the question as to whether that's important.

DR. JOHNSON: John, you made statements that I thought were very interesting, and I thought, since I'm working with ecosystems, perhaps I should give a little bit of my philosophy. I think we have a mixed group of representatives here, and I'm very concerned about how our data are interpreted. First of all, I'm with the Fish and Wildlife Service. By training I'm a microbiologist who suddenly became interested in metabolites and wondered what happened to them, and so I was drug into food chains, and I was never clever enough to figure out how to get out of it.

But John mentioned one term here -- phrasing that struck me -- and that is that we should consider everything in human terms. I think this is rather a dangerous philosophy. I really think, when we begin to look at ecosystems, we cannot isolate man from the rest of his environment, and frankly I look at man, with the small m, in relationship to other species. And, I think, if we tend to think of man only in terms of the ultimate species and ignore the ecosystems and the other species that are involved in ecosystems, we're headed for trouble.

There was a second statement, that we should develop an ecological hierarchy of different ecosystems. Certain systems are not as important as others. I really don't feel that we're at that level of sophistication that we can really decide that this particular indicator or that particular indicator is of no importance, and I think that at this very early stage if we begin to develop these hierarchies, we're in very serious trouble. Therefore, when I look at the particular studies that we are involving or developing, I think that they are merely indicators of possible effects on aquatic life -- I kind of like the term "red flags" -- and they may help industry or may help us as far as a particular type of compound -- red light, stop -- but no farther than that.

But I think, as we deal with the public even on this, it's the Judeo-Christian concept of really why we're here, and where we have this major clash right now is how do you deal with the environment? Is that a stopping over place? I'm not trying to preach this, but it's something with which we have to legally deal. Or are we considered a posteri? Are we considering 2250 or the year 3000, and how do we handle these resources?

When I see the words "human terms" underlined, I think that is a very dangerous thing. I think that's all I had to say on that particular point. I have other things when we get into other data, but just philosophically I think this is a very important point.

DR. BUCKLEY: In my view, man is the center of the universe; he is because he is the thinking organism who is doing the worrying about it. Now in no sense does that mean to me that I'm not concerned about transgenerational effects. I worry about my offspring and my offspring's offspring, ad infinitum. And I'm a firm opponent of the use of discount rates in the process of making decisions as they apply to biological things. So in no way am I meaning to put this down. I have a nagging feeling that all species in one way or another are important, if I'm just smart enough to understand how.

So when I talk about "human terms," I'm really trying to say that you people who are biologists, who are scientists of other sorts, who are worrying about this, owe me as pseudo-administrator some understanding of why some of those things are important. And it doesn't mean that they aren't. Furthermore, we can talk about all the rest of these living things as, if you will, a life-support system for man; they are important to us.

I'm not meaning for a moment to put down the significance or importance of esthetic considerations in here either. You know, I want the air to be clear so the crest of the ridge is crisp when I look at it. That's just as important to me as many other things are. Other people are hung up on different kinds of things, but you can't measure them all. They don't all have a physical or measurable biological response, and that doesn't make them unimportant.

So I just want to state my position on what I mean by in human terms, because I don't mean it in a narrow, immediate sense. I don't mean if you can't fish for it, if you can't hunt for it, or if you can't eat it, or if you can't take pleasure from looking at it, that it's of no importance. What I do mean is that all of these things have a significance to man, and to the degree that we can, we ought to be explicit.

A second point I wanted to make and perhaps should have made before is the whole business of decisions. One of the things that a lot of us fail to recognize is that we in EPA make these on kind of a time schedule. The guy who ends up having to make the decision often has to make the decision on the basis of what is known. Now if he decides not to make the decision, he has just made a decision. If he says I don't know enough to decide, in fact what he has decided in positive terms is that the status quo is all right, and that is just as positive a decision as the other. There's no way you can avoid making decisions.

Our job is to try to get at least all of what is now known to the guy making the decisions. If we've got some evidence that says, "Look, the world's not likely to fall apart tomorrow, and by tomorrow I'll know this much more, so why don't you let it rock along until tomorrow, and I'll tell you some more, and then you decide." That's reasonable, but you need to give him some basis for holding off. You have to remember this character of the decisions, and often, it seems to me, we do forget them.

DR. VAN HOOK: I've got a comment on doing business better. It would be beneficial to those of us not in EPA but under either a contract from EPA, ERDA or RANN, if there could be a little better information dissemination on just what projects are in existence. I just became aware of the Substitute Chemical Program a month ago, and that's my fault, but I'm here to find out what's happening in that Program relative to microcosms. There are other sectors of EPA that are doing model ecosystem work, other sectors of the Government (NSF), and it's difficult to find out what's going on in a timely manner.

If you wait on the Smithsonian scientific information exchange, the projects are over, buried, and dead.

DR. BUCKLEY: Yes, and you might not put the good ones in there anyway.

DR. VAN HOOK: That's true. I just make the comment that it would be nice to come up with some mechanism to let the people around the country know what's going on. Let me give you an example, and I don't want to flag RANN as doing it right, but we put together a trace contaminants abstracts and trace contaminants directory for RANN which tries to keep the people around the country informed as to what projects are ongoing relative to trace elements, and it's been fairly successful. There is now movement to get one going on organics, but it would be good if we could all find out what's happening in a timely manner.

DR. BUCKLEY: The point being addressed is that there are a number of people in other agencies, universities, and industry who, for different reasons, are funding and actively pursuing these kinds of model systems. Somehow or another, it hasn't coalesced yet into one of these invisible colleges where all the practitioners know the others. Maybe it's not the kind of thing which can do that. And I guess I would certainly tend to agree with Dr. Van Hook that some kind of a newsletter or reporting service or other informal thing would be useful.

All of us have a certain secretiveness on parts of this, where we're reluctant to give away new ideas, at least until we've got something underway. On the other hand, there are beginning to be so many of us who have some of the same forces driving us, I really think that we ought to think about the possibility of an informal newsletter, an abstract bulletin, intermittent reports.

I remember, for example, there used to be a PCB newsletter. It never had any status at all. I guess it was the Duluth lab that sort of assembled all this stuff and mailed it around to anybody who wanted it. It wasn't a publication, but it did, in fact, give current results of what was under way. It began as a result of consensus, a recognition that it would be useful, and it stopped, not by directive or anything else, but it just didn't seem to be useful anymore. It might even rear its head again.

But the point I'm making is I think there are a lot of ways and I would hope that, if we agree that's a useful thing, we ought to try and structure some way for it to happen.

DR. EATON: I don't think that all the people involved in the Substitute Chemical Program have a good idea at this point of exactly where they are in regard to the state-of-the-art of micro-ecosystem testing and applicability. There might be a need for something one step beyond a newsletter, such as a summary of the state-of-the-art of this area, that would attempt to interpret the significance of what has been done.

It seems to me that after one year we should have progressed to the point where we are well informed about what various people are doing in this area and that this should be the basis for making some decisions about where we're going, and I think it might be very helpful to have the information summarized.

DR. BUCKLEY: I think it might too. The only reason I'm reluctant to speak to it is that I'm not sure who to point the finger at or how to get it done. Let me make another observation, and that is that, because of the interest of the Office of Toxic Substances in this set of questions, we went out of our way to make sure that those persons as well as those who were going to be working with them were present at this symposium. I suspect that we have about as large a group of people who are working on or associated with these model systems assembled in this room as we're likely to catch under one roof in the near future.

Maybe one of the things that we all might resolve to do before we leave is to sit down and write a paragraph about who we are and what we're doing and which systems we're using. And I don't feel that those things that I've had to say necessarily ought to clutter up the record for all time. I'll willingly give up my remarks for a kind of informal, partial, incomplete directory of "who's doing what," if you'll cooperate by providing a very short statement on your work by the end of the session this afternoon.

I think it would be helpful from industry, to the extent that they feel free to disclose this, and certainly if there are aspects of it which you don't feel free to disclose, but there are others which you can, I'd certainly be anxious to see those. May I also point out that one of the things that you can do in this way, and its hard to bring yourself to it, but it's very helpful to know who's tried something and failed. It really saves the next guy time. We in Government laboratories, in EPA in particular, practically never get around to doing that.

In those laboratories that report regularly to their colleagues and the scientific literature through standard journals and so on, that tends to turn up. Where it doesn't turn up is in internal reports and so on, because what you're looking for there are positive results. I suspect there's a lot of information on how not to go about this business which people know, and were they willing to share, would be very helpful to others somewhat newer to the game.

MS. SCHUTH: I wonder would there be any value in having people submit information to someone (perhaps me) and I'll copy it and distribute it to anyone who's interested.

DR. BUCKLEY: Yes, I think that's a very good point.

COMMENT: Perhaps you might have the participants stand and give their names, addresses, and a couple of sentences regarding their work in the field. This would be a quick and easy method of recording this information now and avoiding further paperwork.

DR. BUCKLEY: Probably not in the transcription but in the reporting end of it. Can I invite each of you who is practicing in this area in its broadest sense to at least put your name and address, institutional affiliation, where you are, something about the system or systems that you're working with and something about the characteristics of the substances you're working with, the kinds of questions you're trying to investigate. If you could do that this afternoon, before the 2:30 adjournment, it would give us something to put together as a starting place.

MS. SCHUTH: What I'll do is have them typed, each on a single page, stapled together and mailed to anyone who lets me know he or she is interested.

DR. BUCKLEY: Okay, we have done something positive during this meeting. There may be some other positive things too, but now we have a measurable accomplishment. Could we now leave the mechanics and turn to the substance of how you go about some of these things, and since I don't know anything about substance, only mechanics, I'd like to turn the microphone back to Dr. Glass and perhaps to Ms. Schuth, who may have some further things to say.

DR. GLASS: I'm not sure I can help much on the substantive matters either, but I think now might be a good time to enter into the discussions concerning how useful these indices are that we've all talked about, how applicable are some of the numbers that we get to the general problem of the effects of these chemicals in the environment, and one question that came to my mind this morning while Dr. Johnson was making his comments concerning microbiology. Is it possible that the microbiological system is somewhat more diverse perhaps than other types of biological systems and that, because of this, maybe there is enough redundancy in that system that you don't need to be concerned about whether or not particular species of microorganisms die as a result of your toxic chemical?

I don't know that that's true. I am just asking if maybe there's some difference between the kind of diversity, for example, that you observe in the micro-community relative to the sort of diversity or other community parameter that you might measure on larger communities. I wonder if there is any merit to the thought that possibly diversity indices are different, depending on the type of community it is.

COMMENT: In determining about microcosm -- we really do not have a microcosm as such, but a modular system -- and we use microorganisms merely as a type of indicator of the accumulation of particles. Matter of fact, we ended up using yeast because we found out it didn't make any difference in the bacteria or algae. We used yeast simply because it would be very readily available to people, and they didn't have to have a lot of background on how to culture organisms or then you get into a raging debate on what organism to use. And so we discovered that yeast were quite adequate.

As a matter of fact, it appears that even autoclaved killed yeast may form the same basis for studying the accumulation of particulate matter, biological particulate matter for this particular value. But responding to the type question, it just so happened that we're quite interested in this area for another reason. One of the major problems that has always plagued administrators, I think, is the interpretation of the degradation of a particular compound. Joe Blow says his compound is just no problem whatsoever, it's readily degradable. And then, lo and behold, we look at our national monitoring program or something of this sort, and we find out that the compound is present at horrendous rates. Something is wrong somewhere.

We find out that perhaps this individual has done a pure culture study or something of this type, which is really relatively nonsense for environmental work, or he may have done a study in sludge, where this particular compound is never going to be, even if you get into this type of ecosystem. So it becomes a question of how do you define this type of a system so that you can do degradation studies?

We have to zero in immediately and not look at the whole world but just look at heterotrophs. This is what we are doing and we have begun work along these lines.

And we do see changes in the organisms, and these do influence degradation. How important they are, we don't know, but there is something that does occur, and that is the microflora during degradation periods do change. What we're interested in is have they changed to such an extent as to change what's going to happen in the environment, so that you now are looking at an organism that in the environment never would be greater than ten to one or two per some unit, and now we find out that, due to the conditions, that this organism is at very, very high levels.

This is a major consideration, and we are going to do extensive work along these lines, just to determine this, but strictly in terms of heterotrophs.

DR. GLASS: Another way to look at it is to ask if the material degrades and not is there a change in the composition of the organisms that degrade it. What you really care about is whether or not the compound disappears over some reasonable length of time.

COMMENT: Yes, but there is another flip side to this, which is another project that has concerned us a great deal, and that is that we have been so concerned with the parent compounds. A good example of this, I think, is methoxychlor, on which Dr. Metcalf did some work. It was a very interesting study, and this has been in the literature repeatedly as a biodegradable compound. It is biodegradable in the sense that it has lost its initial toxicity, and it had been hydrolyzed, but we still see the ring structure, and we begin to ask ourselves what's happening to these compounds, these persistent things, such as pesticides, that have sat around and no longer have the apparent biological activity. We are beginning to look at these in terms of their effect on geochemical cycles, because we feel that perhaps we're overlooking these and that we may be having marginal effects of one or two percent, which have an effect on ultimate productivity as far as our resources are concerned.

COMMENT: The whole issue of whether the metabolites are too from the breakdown product.

COMMENT: Perhaps this shift in materials, this shift in certain colonies, certain types of organisms, may influence the geochemical cycle, particularly nitrogen, sulfur, etc., that may have a very important effect on productivity.

This particular topic you're discussing relates to bound and conjugated pesticide residues, which the Vail conference was principally about, and the bioavailability, if you'd like, of conjugates or bound or unavailable type materials to the environment. This not only includes plants, earthworms, the whole mix, whatever was available for testing, and I think the crux to the whole problem is it was established, I think, that almost everything that we put there is going to still be there in some form or other. It's not just going to go to CO_2 and water very quickly.

The whole thing is the significance of something in the environment which we may call "unnatural." If it's not bioavailable to a number of different test species that we determine, how significant are those results? I think that's the crux to the whole problem, that we have to set some criteria and test species here, and if it's not bioavailable as we define it, then how else can we go until the state-of-the-art continually progresses?

But if there is something there at some level, as we determine by radiolabeling techniques, and it's there 100 percent, but in some other supposedly unavailable, nontoxic substance, yes, we should be concerned about it, but the significance that we place upon it may be a much lower priority than something which is more available.

COMMENT: I suppose it would matter what compartment or component you found this material residing in. If you found it bound to a soil particle, for example, you might be less concerned about it than if you found it in some protein compound in an organism.

COMMENT: For instance, in relation to the soil thing, Cleve Goring and John Hammecker of Dow had gone through an elaborate mathematical model as well as through practical applications of a large number of chemicals being bound to soil, and discussed the relevance and significance of things that are occluded, bound, that you can't chemically seemingly get out of the soil. Now it's there, and it might be there for thousands of years, millions of years, but is it really going to affect our environment, either us or microcosms, the whole works? Philosophical question.

QUESTION: I'm wondering if we can get back to diversity just for a minute. It's my opinion that some methods for determining diversity have progressed a little bit further than analysis of the significance of them. This is opinion based on practically no experience. It's also my opinion that it is an obvious tool in cases where there is gross contamination and that changes results that are easy to see and interpret under those circumstances. I'm wondering specifically if anybody would care to enlighten me a little bit regarding the sensitivity of diversity as a measure of effect of substances on the environment. It's strictly out of ignorance that I'm asking that question.

COMMENT: There have been several review articles in the last five or ten years that look at different types of indices and test their sensitivity, mathematically speaking, and which apply different indices to the same data set and different data sets to the same index, etc. I'm not sure that there is a clear pattern that's emerged from those reviews, other than to say that you don't always get the same conclusion from two different indices applied to the same data set, for example. If you had one set of data from which you could calculate a diversity index, you calculate two indices, and you might discover that you come to conflicting conclusions or at least different conclusions.

QUESTION: Is that the same thing regarding sensitivity? It seems to me one of the big drawbacks at this point in time regards our knowledge about the sensitivity of diversity in terms of their being able to make evaluations regarding effect.

COMMENT: I think I understand your question. I just don't know the answer.

DR. KENAGA: We talked yesterday a bit about the benchmark concept summarized by Dr. Riz Haque of EPA. That includes benchmark chemicals and benchmark test methods, the way I look at it. Dow has done quite a bit in this area for a good many years. You've heard our researchers' names mentioned on several occasions. We feel that this has been a very useful concept for us in research, if nothing else. In research we found, for example, that octanol/water partitioning coefficients and soil organic partitioning coefficients and fat residues in fish have a very good correlation in coefficient numbers. Bioconcentration factors are calculated on the basis of those techniques, and so they have a lot of things in common.

Now there are several ways to go about studying environmental pollution. One is by studying a minute detail for your entire life. Another way is to determine the important ways in which a chemical dissipates in the environment. The study of the physical and chemical properties and the proper method of comparing them with other chemicals gives you a great lead to that in research.

I have been encouraging this latter concept for a long time through my publications. I feel dedicated to the fact that this is a useful tool. However, government regulations are what we all have to face in industry. For example, in the appendix to the new Guidelines there is a test method on octanol/water partitioning coefficient and there are numbers that are suggested -- I think it's 100 -- as to whether something might be an indicator of bioconcentration from the octanol/water partitioning coefficient.

However, this is not one of the test methods suggested in the Guidelines itself. So there isn't a matching up of those numbers and test methods in the Guidelines themselves. We have tried to make use of partitioning coefficients in trying to register products and maintaining that these coefficients are correlatable and useful as indicators as to what might happen in different segments of the ecosystem.

However, we don't get very far with EPA in using those partitioning coefficients when there's nothing in an EPA checkbox that says that you have to do this test method, because they don't seem to know how to interpret them. And we're hoping that, if we do establish within EPA that those benchmark characteristics and others mentioned by Riz Haque are valid, then we can use them in our arguments as to which test methods need to be done to answer the questions as to distribution of pesticides in the environment.

I realize that it may sound to some industry people like we're being stuck with test methods which are not the same as our own, but I would submit to you that nearly all of the Guideline test methods are becoming that way, and our only hope is that we encourage the use of test methods that are valid and are really meaningful. In the present test methodology on model ecosystems there's only one that even has an EPA test method guideline to it, and that's the flowing water bioconcentration test. Another one that's inferred but not written out is the test method where you treat soil and put the soil in water and then have the catfish exposed to it about a month after the soil has incubated the compound. But that's not really in the method. We've been talking a lot here about many different model ecosystems. It would be frightening for industry to be faced with four or five different model ecosystems to have to use in the registration process for each compound.

That's not to say the research isn't needed for model ecosystems. And I certainly would be the last to discourage that. I can see that terrestrial model ecosystems require different organisms than aquatic ecosystems. The partitioning of these compounds in environment are all subject to water, which is the major liquid in all ecosystems, and to fat, which occurs commonly in plants and animals. The general partitioning trend of the chemical in the environment may depend upon the ratio of fat and water to each other. Use of the data that we have on residues in fat tissues in pesticide-treated cattle or other animals that we usually have had to submit with the registration of the pesticide supplements partitioning coefficients that occur in fish and other ecosystems.

There are many ways in which the total input of the information gained by use of test methods named in the Guidelines lead you to the same direction and the same conclusion. Industry certainly hopes that with the Toxic Substances Act coming up for passage we don't have two regulatory groups with two different bureaucratic sets of test methods for each evaluation (i.e., fish and wildlife, physical and chemical properties, etc.).

Now I've been assured that this will not happen, but I will wait and see. I laud the people in the lower echelons who, I'm sure, believe that there should not be, but I'm fearful of the upper echelons.

COMMENT: I have two special comments. One was in relation to Ms. Schuth's work about the octanol/water partition coefficient. This could be a reliable indicator of bioaccumulation, but I think that using this coefficient in the fate studies may lead to some misleading conclusions, because other factors like specificity, localization at certain sites, degradation, excretion, play a very important role in determining the toxicity of a compound. So I think we should be somewhat cautious there in applying partition coefficients to the fate studies.

And my second comment was in relation to Dr. Hayashi's work which he presented yesterday in using the in vitro system as a screening method for detecting the toxicity or the mechanism action of a compound. I think using systems such as chloroplast, isolated chloroplast or mitochondrium, or cytochrome P450 and exposing them to a certain chemical in test tubes could be very misleading.

I think if we want to use this in vitro system, it should be in vivo exposure, followed by in vitro testing, and I'd like to hear comments from some other people along these lines, because it has been pretty well demonstrated in certain pesticides, for example, a very nontoxic compound like PCNB. If you take a cell-free preparation of cell membranes and feed the in vitro system, this compound will show you all kinds of membrane alterations in the cell because, being a sulfidal compound, it reacts with the proteins, amino groups. On the other hand, if you feed this thing to animals, apparently you're not going to see anything there. This has been already demonstrated with other pesticides like Atrazine. All we know is that the corn is very resistant and soybeans are very sensitive.

If you expose the corn plant and soybean plants to the herbicide and then measure the photosynthetic rates in the leaf discs, you will find that corn is not inhibited and soybeans are very sensitive. On the other hand, if you take the chloroplast of corn, which is a resistant species, and expose this to the chemical Atrazine, you will see almost 100 percent inhibition.

The point I'm trying to make is that this work entirely dealt with the in vitro exposure, and I think we should try to make the interpretation very carefully, and this is a very hazardous approach.

COMMENT: I have to explain something about our research project. First of all, when we evaluate any herbicide mode of action, we have to think about physiological result, physiological phenomenon, either one as the result of biochemical reaction. First, we are taking the various kinds of biochemical reactions, both in vitro and in vivo systems. As I reported yesterday, the time was so limited, so I just picked up the important data, and right now we just concluded what kind of method for evaluation of a herbicide. We are concerned about the best way to use whole, intact plants and also some in vivo systems. If you have seen my report, right now we are using some susceptible plants, intact susceptible plants, actually soybean plants, to evaluate herbicide, using oxygen uptake.

As you have seen in the slide of the 15 herbicides, 12 herbicides are effective to the oxygen evolution. In order to evaluate the mode of action of a herbicide, the best way is to use the whole intact plants. When we use the corporal system, we have a lot of troubles. Once we were thinking about evaluating the mode of action of a herbicide using a protein pattern on a corporal system, but it was not successful. Every plant species has a different response to the various herbicides or pesticides.

Right now we are using the whole, intact plant. Of course, before we made the conclusion, we used in vivo and in vitro systems of chloroplast and metachondrial systems.

COMMENT: Yes, I think we should try to distinguish if the mode of action is the mechanism by which a chemical produces its toxic effect, irrespective of whether it reaches the site of action or not. But what I think that you're trying to do is to find a sensitive screening method.

This is an inherent mechanism. If a plant is inhibited by certain biochemical reactions, if we go by the whole plant, like a resistant plant, we will never be able to see this mechanism of action because by the time it reaches the chloroplast, reaches the site of action, it will get degraded into a nontoxic compound. The only way you can see the mechanism of action is by working a system in which we have eliminated the factors of degradation, eliminated the uptake, transportation.

This is a screening method for determining toxicity of the compound, not a screening method for determining the mechanism of action of a compound. That's my whole point.

QUESTION: Do you have any idea of what kind of methodology we actually have to use?

COMMENT: No, what I was saying was that if you are going to use a screening method using chloroplast, industry is going to use chloroplast or metachondria of P450 as sort of enzyme system, isolate the enzyme system. I don't think this is going to give us the meaningful information, because under those circumstances you're exposing the system to the highest concentration of the chemical, which apparently will never happen in the whole animal or the whole plant.

If you establish a system in your research and then on that basis you recommend it to industry or to universities saying, "Look, we have this chloroplast, this is our system, you go and screen all your new chemicals in that," I'm sure you're going to come up with very misleading information based on these studies because there are many, many compounds which apparently don't produce that. If that was the case, we won't have species sensitive to herbicide and species resistant to herbicides. In the literature, most of the compounds are in plants which have the differential sensitivity or resistance, a lot of times it's due to the differential uptake or differential breakdown of the compound.

So this is the thing that we have to keep in mind, that we should try to keep away from isolated chloroplasts and isolated metachondria. If you want to do it, you should expose the whole plant to the chemical and then try to isolate its chloroplasm, metachondria or whatever other organisms you want to work with.

COMMENT: I think probably you misunderstood that first we use the whole, intact plant. You remember that we put the whole, intact plant in a chamber and detected carbon dioxide, using the aniode gas analyzer. Why are we evaluating carbon dioxide stimulation and the respiratory effect? Because first we have to evaluate the mode of action of the herbicide using the whole, intact plant.

COMMENT: That's the point I'm trying to make, that you cannot study the mechanism of action in the whole plant, because you don't know what you have done to the pesticide. You don't know what is producing the toxic effect.

COMMENT: We talked about a practical test, and you're talking about a mode of action.

COMMENT: I still maintain that it's difficult because you are adding other factors because one doesn't know what has happened to chemicals before they produce an effect -- maybe the chemical has been converted into compound A or has been converted into compound B, and that's the one that produces the effect.

COMMENT: I still am not clear about how to evaluate the mode of action.

COMMENT: You should isolate your system.

COMMENT: You still disagree, because we have done some experimental work using isolated chloroplast. This is not of practical use and I think I'd like to discuss this problem with you. Too many factors are involved; for example, the surface of the chloroplast is directly touched to the herbicide solution. If we evaluate the herbicidal mode of action -- those data are not of practical use.

QUESTION: If EPA is charged with regulating the occurrence of these chemicals in the environment, the distribution, the fate, and the ultimate effects, and if industry is also charged to produce these various materials, free enterprise, and all that sort of thing, how do we, the contractors like myself and others who work for EPA, get the information from industry that helps us scientifically devise a system that supposedly we're going to build to give to industry to help them evaluate chemicals?

Now there are a number of grants that have been let where the contractor is told help devise a "sensible screening tool" that industry can use. How do we know industry's going to accept it? EPA obviously could force it, but that's not the best of all situations. And my question is, how do we get EPA and industry together to find out what both needs, so we could get the happy compromise?

COMMENT: You don't want to reinvent the wheel.

COMMENT: Exactly. They've done a lot of things that we're unaware of.

DR. BUCKLEY: I guess there's no clear-cut answer, obviously, to what you've just asked. One of the things that EPA tries to do in its regulatory action is to be as open as it can along the way. Now I understand that we're criticized for both extremes, being too open and, on the other hand, never talking to anybody. I submit that this meeting, for example, is one opportunity to exchange views or to begin to understand what other parts of the community are doing. It seems to me there is a second stage. Let's assume that the object of this whole exercise were to erect a system which could be imposed by the Government, followed by industry, with the object of net gain by society.

One way of doing this is for EPA through contractors, by whatever mechanism they can, to assemble information and to put this out in the form of a proposal for comments.

We're now in a position where we don't even have a law (the Toxic Substances Act). We can still shape that. But there will come a time when there's some kind of legislation.

One of the things we might consider is an "advanced notice of proposed rule making" in which we set forth a proposal, follow it with a series of public meetings in different parts of the country to bring together people from all sorts of interests to discuss what's impractical about it and why you can't do it that way or, conversely, why you must do it that way because there's no other way of getting at the information.

The usual practice in EPA is to issue an advance notice of proposed rule making to try to pick the biggest of the bugs out of the system that way. We then go forward with proposed rule making in which people realize that we are being serious about it, and sometimes elephants instead of bugs come walking out of the woodwork. And finally, taking into account the comments, we publish a set of regulations which have effectively the force of law and which have been looked at by everybody. That doesn't mean you all agree on it.

COMMENT: May I suggest that perhaps there's another way which has been utilized recently very effectively, I think, and that is through the American Society of Testing and Materials, whereby all segments of those people who are in a position both to use and regulate these things have the opportunity to agree before it is put into the rule making process and which gives a lot more ample time for discussion of the pros and cons of the various methods, and theoretically requires consensus agreement on the methods prior to the time that they are published and made available as being another alternative means of accomplishing some of these.

DR. BUCKLEY: I think that's a good point. I think to the degree that it can be used, it's a very useful process. I submit that what's going to happen in practice with the Toxic Substances Bill, however, is one day it will pass, and all of a sudden there will be an urgent drive to get guidelines to industry, on the street, whatever they're called. They may have the force of law; they may simply be guidelines; or they may be something in between, but somehow or another that's going to happen.

We've got a few months ahead of us yet in which we can still do some sensible things jointly, but the closer we get to needing to put out something that people must do, the tighter the deadlines, the greater the difficulty in doing that. And I would just urge that we talk as freely among ourselves as we can. As Gene Kenaga has pointed out, industry has got a wealth of information that they have worked with over the years with the deliberate intent in the pesticide area, for example, of trying to find what are those things that will be good to kill pests and won't also do a lot of bad things at the same time.

These are, in fact, screens because there are innumerable potential chemicals, some of which are more promising than others. As a practical business matter, they've been trying to decide which ones to develop. And it's obvious that they don't want to get to the end of the scheme and discover that this is fine and everything, but unfortunately, it makes people drop dead or, for some other reason, is just totally unacceptable. Somehow or another those of us in academia or in research institutes or in regulatory groups or whatever, do really need to extract whatever we can from these systems.

DR. KENAGA: I think industry screening techniques can be used for evaluation of many compounds. Screening is a word we've used here a little loosely. I think it means different things to different people. In industry screening is when you take a compound and, if you've had it for the first time, and you submit it to a lot of test methods to find out whether it's going to filter biologically out and become a viable pesticide candidate. And in that sense industry has many, many screening techniques that we've gotten from anybody that looked like they're practical. We don't care where they come from, as long as they give us an answer that is practical.

We've had a lot of experience in evaluating techniques or test methods that are misleading. So we're a little allergic to those. And one thing that a company really hates is to have the first compound tested on a new test method, which nobody knows how to interpret or use, and then somebody publishes data and says what a terrible compound it is.

QUESTION: The subject matter came up before about test indices which are ultimately the objective of looking at model ecosystems and, whether or not you look at one type or another, at least have some common ground of comparison. I don't personally do a catfish study. I have it done for me. I was involved in assisting on some things for registration of compounds. I get back numbers from which you can take and calculate the bioavailability, rate of degradation indices. How do we judge in industry the meaningfulness of these indices, and what importance do we place upon them?

Are there guidelines that define some numbers which are acceptable or unacceptable? Obviously, it has to depend on what kind of compound. The compound itself may have different toxicology and other things like that that make it completely safe, so you'd have a higher number. But are there guidelines or numbers so you can judge yourself a little bit?

I go back to the data, I start to assemble it. But we may continue on another six months or a year's program before we submit the whole package. Maybe that's an alarming thing right when I get back and I look at it and I say, "Oh, oh, we'd better stop right now." Do we have to wait to get to the Government so they can have them review it so they can come back to us a year, a year and a half or two later, and say forget it fella, you're out of luck now? Is there something that we can assess the situation on?

COMMENT: If you take the magnification values that have been derived in the terrestrial model ecosystem, and you go to Illinois Farm Ponds, you find that the values fall right in that order of magnitude.

I don't think the absolute values are what you're interested in, it's the relationship, as you point out, between compound X and compound Y which is known to persist and has already been shown to be persistent.

DR. BUCKLEY: I wouldn't even want to draw the analogy. Although the Guidelines suggest what you might do with another chemical, a given chemical X which is in the same family as another chemical, many factors are going to be significantly different. There can be a fair amount of difference, the distinctions of metabolism, other factors.

COMMENT: Use patterns.

COMMENT: The whole works. So that you can't draw the parallel. You might have a biological accumulation, and something of 200 which you normally define as being bad, whereas another number may be 75 or 50, and that may be just as bad. It depends upon the chemical.

COMMENT: One must take many, many fragments of information and put them together before one can make a decision. I know a compound in particular that will show a very high accumulation in fish, but it is readily degradable in hydrosoil. If you were to take fish alone or take the octanol coefficient and look at those two, you would say that it's a no-go compound. But if you were to look at the degradation element, you'd find out that it's rapidly degraded. This changes the story. You must take these and put them all together, and I think we all agree on that.

DR. BUCKLEY: Let me take what you said and push it one step further. If you took that octanol number and found it was high, what that says to me is I'd better look at fish if it's going to get in the water. On the other hand, if it's low, I have a pretty good feeling that it's not likely to accumulate in the fat. That doesn't keep it out of the bones, it doesn't do a number of other things. It doesn't answer all questions, but it does give you a clue that you need to know more. And you, as an industry guy making judgments, are just as competent as those who will make the decisions in Government in thinking about what the next question is and whether you need to pursue it or not.

Another thing that has been emphasized here two or three times and probably needs to be continuously reiterated is that if a substance is not particularly toxic and you don't care if it's around at a part per 1000, then accumulation may not mean anything. On the other hand, if accumulation will result in levels within an order of magnitude on the range where you think there may be a problem, in that case you're going to be a lot more careful. I think it comes back to this word screen.

Again, I don't think any one of these is definitive, but I think they point you to other questions you need to ask and, hopefully, if the screen is worth anything, it will save you answering some of those questions.

QUESTION: There's one more aspect to that question posing that nobody has approached yet. As you're running the test with a given compound through everything that you can stop at, at that point you've determined that you have enough information to decide that it shouldn't be developed any further, is there some way to keep in contact during the development process to allow you to save money?

MS. SCHUTH: That's a business decision.

COMMENT: Yes, I don't think it's appropriate for Government.

QUESTION: If that is not the question, I will retract it, but is that part of the question? Did I interpret you correctly as saying that?

DR. BUCKLEY: It seems to me that that is a business decision, and just like Government decisions, there are two sides to it. If the promise in terms of bucks in the pocket from that compound is extremely high, you may be willing to accept some results that you wish hadn't happened and then pursue it to a considerable extent beyond there. On the other hand, if this is an exploratory thing and you don't see the potential for a big market with it, and it's got a couple of strikes against it in terms of characteristics that will be hard to live with, you'd probably drop it. I don't think we ought to get into it.

COMMENT: There you are at the business end of it, in terms of the economics of can you make enough out of it in that way to make it worth pursuing and digging up the information needed.

DR. BUCKLEY: I've tried twice and I'll try again. It seems to me one of the things I'd like to know is, are these any good? I don't mean indices, I mean model systems. If they are, what are they good for, and more to the point than that, if they have some promise, what can we do about them to make them better? Are there some questions that we've overlooked? It seems to me we've been all around the question and we've tiptoed up to it a half dozen times, and nobody has been crude enough to tell Jim Gillett that his system was too complicated. Nobody was crude enough to suggest that the terrestrial one might be too simple.

What I'm interested in from you people who are working in this area accumulating data is some feel for things that would be better, or things that can't be done, or things that are worth looking at that you haven't gotten to yet.

I saw an aquatic system yesterday and I was curious about atmospheric inputs and outputs. I wondered if you couldn't use that same system and extract an additional piece of information by controlling the atmosphere over it. I'm reminded of some work that EPA had done in our earlier days -- not all that much earlier either -- when we were obligated apparently to work on things that were in water. If it left the water into the atmosphere, then it wasn't considered our problem. It was somebody else's problem. Somehow or another, it's all one environment.

Doesn't somebody have something to say on both the shortcomings and the possibilities? Are there some imaginative ways we can tie things together that we haven't yet? Are there some questions about the way things are now being done?

COMMENT: Jim Haggar's model which is under development was a tool of the design to model the microcosm itself in the hopes that we could do something like a sensitivity analysis on the model and determine if there were aspects of the microcosm that could be deleted and not lose too much information. That's in a state of development at the moment; it's not completed, but I think that's one possible approach.

COMMENT: It seems to me that Dr. Johnson in his food chain studies has been dealing with some of those questions, but not necessarily always in the same box. Sometimes it's several boxes, and you get to a particular point and you move the experiment from that box to another. Maybe you don't literally have to move them, but now that you know how far it gets there you can find other ways to look at it.

COMMENT: For several years when we were thinking about how we can put together some system to test compounds, we asked would it be degraded in an aquatic system, would it be accumulative, would it be transported, and could it be done for under $5,000.00 or under $500.00, or some value that seems realistic, particularly if a company had 18 or 20 different compounds, or if the Agency had a particular herbicide or insecticide that it felt was very important in managing certain types of resources.

We came up with mathematical models -- I'm using the word model probably in the broadest sense. I apologize for that, but we felt our particular system could not be put together in one unit because we could not control. We had a problem of controlling biomass ratios in relationship to nutrients, and so we came up with what we called modular, or used it in this modular fashion. We can look at each segment by itself, independently of the other, and then intermarry these at our own convenience. We felt that we could very accurately study uptake, we could very accurately study degradation, and we feel that this sort of thing works reasonably well.

One other thing we felt was a weakness of microcosms systems is that it's very difficult to follow the nutrients. When you're feeding a particular organism fish or something of this sort, are you feeding him at a realistic level? Are you starving him or are you feeding him at such a rate that this is unrealistic? With our system we can calculate these or we can adjust these. Then you also know what is happening as far as the nutrients are concerned and so you have many handles. Our model was taken apart.

To go back to your question of how good they are, we feel that they are only as good as the people who operate them, and maybe in two more years with all this testing Carter will find out if they are reproducible. We do feel that they offer, in conjunction with other data, red flags. They offer indications of probable difficulties.

We feel that an index is useful because it does help those persons in the hierarchy who have to make the decision when all the data are gathered, and that's usually either a vice-president somewhere along the line or perhaps an administrator who is a gifted scientist but who may not be really up on all of the idiosyncracies and the difficulties.

Thus, if we give them some of these values with the proper guidelines along the way, that this means so and so, we feel that these numbers could be of some use. We have based all of our accumulation data on DDT. We know a great deal about DDT, its ubiquity in the environment. It is quite easy to work with in the laboratory.

Therefore, if I say a compound has an index that's, say, based on DDT in unity, an index of 110 -- we'll say DDT has an index of 100 -- this gives you some idea that this is a problem. If it has an index such as some of the triazines -- these have 0.001 or something like this -- it indicates to you that triazine as far as accumulation in these aquatic systems is essentially no problem. This is the type of information you can come to, but it does need to be used in conjunction with other data. The index, we feel, has value, and I like it. If you can come up with better reasons for approaching it from some other way, fine. We are constantly asked what does 75X mean? Is this bad, good, or indifferent?

The answer is this is a value. It isn't good or bad, it has no value. Scientific data are not good or bad. It is a number, and 75 or 1,000X could mean under that circumstance, it has this value. Under environmental circumstances, in use in conjunction with other data, it may really mean that nothing has no value.

QUESTION: You use the word index as sort of a single parameter type index.

COMMENT: Yes, but it could be multi. We're using it as single right now.

QUESTION: Bioaccumulation.

COMMENT: Yes, right.

QUESTION: It seems to me that for an index to be usable you would almost have to have it be a multidimensional index.

COMMENT: Yes, and this is the next thing I would like to bring up. The great problem is how to weigh these. The Metcalf and Sanborn methods use an index based on degradation. You could use one based on LC_{50} or not index, but all these fragments of data could be put together and you could end up with five or six or seven of these, and come up with a particular numerical value which would tell you something about that compound environment.

I think of the analogy of the old heart. Have you ever seen these at the doctor's office? As I was looking at it, I thought this is marvelous because it was indicating whether you'd have a heart attack, whether you're in bad shape, such things as your age, the number of cigarettes you smoke, whether you have a high lipid diet, or how much cholesterol you have in your blood. You have all these values. These are arbitrarily given values, and you add these up. There is a little index which says if you're within such and such values, you're going to die before you leave the office.

This sort of thing might be useful in some of our compound problems, but I don't know how you'd weigh them. This is where the argument is. How would you weigh an LC_{50} of a part per billion which is a range for the organic chlorines such as DDT, versus a compound of the organophosphates which perhaps would be 100 parts per billion, and then go into things as effect on reproduction? How would you weigh that? This would be the ultimate. No one at our laboratory agrees with this; they think it's great but then we get into the arguments over values. But that's the ultimate.

DR. BUCKLEY: I worry a little bit about questions of simplification and oversimplification. It's clear that what we want out of this whole situation are simple ways of evaluating complex events. What I hope we don't end up with are simplistic ways of evaluating these which really have no particular merit associated with them.

I don't mean that as a put-down to indices, because I submit, for example, that one that we all use in one sense or another in our daily lives is an appreciation of what the GNP, the gross national product, is. We may not know with precision but you have kind of a gut feel that when it goes up, certain things are happening, and when it goes the other way, there are certain things associated with it. It's a very arbitrary kind of thing, put together out of those things which are conveniently measureable. It has no intrinsic merit; the ingredients are not necessarily all causally related, or relatable in ways that we can even specify, but they do tell us in a single number that things are getting "better" or "worse" in terms of our national economy.

I submit that we may, with the passage of time, find that it doesn't tell us at all what we want because at the point in time GNP was developed, the presumption was that continuing growth was the mode that this nation and the civilized world would be in. If we change our goals and decide that stability of some sort or another or some lessened growth rate is the desired mode, then the way we use the measure may be very different. We may both want to change the index and perhaps change our interpretation of it.

I think that this same kind of thing would apply to the indices here. I'd like to see if we can turn now from this to the physical experimental systems. Let me ask a question of Dr. Johnson. What questions can you answer with your food chain model? What things that are important can you not measure with it?

DR. JOHNSON: We feel that we can reasonably and accurately reproduce some values as far as uptake from the aquatics in microorganisms. In our total food chain we attempted to look at three trophic levels: a microorganisms trophic level, if there is such a thing; a filter feeder; and a fish.

These represent distinctive niches, and we hoped to interpret the uptake and determine what the plateau levels were over a given period of time. We're hoping to determine what happens to the compounds -- were they degradable, is the nature of the compound being changed now, or do you get into somewhat of the Pandora-box syndrome type of thing? You are able to know what happens at a trophic level that you're interested in, and for us it was the fish and so we can look at those things.

We have about five areas that we can look at independently or simultaneously, and we can get both qualitative and quantitative information. One other thing which we haven't emphasized, but it's quite important, is the elimination of the compound, that is, if it is picked up, how long does it stay in the particular organisms? We are interested in the half-life of the compound.

Those are the bits of information that come to mind readily, and which we looked for. You can come up, if you like, with an ultimate index if you want to compare it or just if you have straight values under these things. You can also quite readily in these systems do all sorts of manipulating with biomass changes. You can stress your animals, you can do temperature studies. These are all the most obvious, but they're relatively easy to do in these systems.

DR. BUCKLEY: This is a more general question, but it's triggered by what you said. What consideration do you give to the way in which this substance might reach the environment? For example, the organophosphorus pesticides are ordinarily used intermittently.

DR. JOHNSON: This is a very important consideration. I would have had to go through every aspect and probably given you a complete seminar of what I tried to do. This goes into what is called toxic or dosage or concentration relevancy. How is a compound going to get there? Is it going to be an effluent? What is its concentration? Is its projected level one that you may find in water?

The one that we feel is the most important, at least that we use a great deal, is based on acute toxicity to a component of our model. We either used invertebrates or fish, depending upon the compounds. This is a very important consideration because it's quite easy to use a concentration, water concentration far and exceeding.

One of the problems is consideration of such things as the introduction of the chemical, whether it's going to be intermittent or is it going to be continuous. This is a very basic thing which some people call static versus continuous. These are considerations with which you have to work. We lean toward being continuous for most of our food chains, but we also have set-ups for statics. The chemical is introduced once, but as you deal with the organochlorines, these get into all sorts of difficulties.

In regard to the other question about things we can't do, I think the difficulty is an integrated thing where everything is all together at once. We cannot do this.

DR. BUCKLEY: Even if your chemical may be introduced only once, the metabolites or some of the other breakdown products might be continuously available to the next trophic level, or the next along the chain.

DR. JOHNSON: Yes, exactly.

DR. BUCKLEY: We haven't heard very much from our industry counterparts in this microcosm business. I'd really be interested in finding out to what extent microcosms and apparatus of that type are in any kind of use in industry. Are there any tests that are either routinely performed on a number of chemical types? Can anybody shed any light on the use of these types of apparatus, outside of the Government or academic community?

DR. GILLETT: There's nobody here from Ciba-Geigy which is the only company I've heard that is doing it.

MR. NEWBY: I'm Loy Newby, Ciba-Geigy. We are not conducting ecosystem research. We are in the process of developing a modular system, that is, our Basel laboratories are so engaged. My concern here is that although the ecosystem work is personally very interesting, when it comes down to a company, we meet with the Registration people (who are conspicuous by their absence here) and essentially the buck stops there. We do a dynamic study, or we do some other aspect of a fish residue study.

DR. BUCKLEY: So you don't have any sort of special protocol or procedures that you send prospective chemicals through and hope to come to some understanding of their possible ecological effects before you entered the registration business?

MR. NEWBY: Certainly the Guidelines and the Appendices list a wide range of studies that we have to do. We go beyond this to some degree, but not in the microcosm or ecosystem area. As Jim pointed out, he thought we were engaged in such research because one of our colleagues from Basel recently visited here, trying to come up with ideas for a system which probably will be a modular type.

You must take into consideration that in a modular system you may get a high degree of accumulation which I'm not always sure what it means, but I will agree with Tom Johnson and Jim Sanborn, it throws up a flag. We have some concern, so let's next look at it from a more realistic standpoint. Let's apply it to the soil, age the soil, then add water and fish, a bottom feeder, determine how much accumulates in the fish, and you may get a completely different story.

DR. BUCKLEY: The point is, you go through those sorts of steps. How elaborate are those steps, or is that something you don't feel free to really go into?

MR. NEWBY: No, I can't go into all of it certainly. We feel when we present our data in an environmental submittal, we have a pretty good idea of what that compound is going to do in the environment.

DR. AMUNDSON: We are not involved in developing microcosms; however, we have used them. We have sent compounds to Dr. Sanborn, Illinois Natural History Survey; Dr. Kearney and his associates, USDA; and to Bionomics for studies of various kinds.

In spite of the emphasis on the development of ecosystem models for the prediction of bioaccumulation, one can get a good approximation of the potential of a compound to bioaccumulate by looking at water solubility, partition coefficient, soil degradation, and animal metabolism. Some compounds which are quite persistent in the soil environment are readily metabolized by animals and fish, so one has to evaluate several parameters.

We feel that the current Guidelines for Registering Pesticides in USA are fairly reasonable, and I feel industry can live with these Guidelines. The important thing is interpretation by the Agency of the data which are submitted for registration purposes.

Dr. D. G. Crosby, University of California, Davis, has developed another index of environmental concern which he has applied to several pesticides. This work will be published by him in a paper entitled "The Toxicant-Wildlife Complex." The formula for calculating the index includes solubility, P values, and toxicity to selected aquatic species. Perhaps it is another way of evaluating compounds as they relate to the environment.

QUESTION: So it is a multidimensional thing?

DR. AMUNDSON: That's correct, and I think the same thing is true with the Bionomics fish accumulation, with Jim's tests in Illinois, or with Kearney's tests at Beltsville. They are indicators, and we routinely send all candidate pesticides through one or more of these tests, just to get some idea of what their problems might be later on.

COMMENT: From your comments I gathered that the new Guidelines are really not that great a change from your current practices, that maybe they're all spelled out in one place.

DR. AMUNDSON: They've been used for some period of time. It's kind of a continuum.

COMMENT: They appeared in the June 25th issue of the Federal Register. There are 60 days through August 26th within which to make comment regarding the content of the Guidelines and Appendices. It's worth everybody's time to look at them and comment if you have an opinion regarding their completeness or content.

QUESTION: I would like more guidance not on just what to do or how to do it, possibly what the environmentalists feel would be appropriate, but also how I can judge and determine the significance of every experiment I do.

Obviously with my limited exposure to things that I'm just doing, or seeing something in the literature that comes out several years later after it's been done, I can only judge from a limited point of view. I'm not asking EPA to make a business decision for me which really is my decision in upper management. I'm not just asking for guidelines that suggest screening procedures or tests, I would like to be aware of the significance of those tests with some handle, qualitative or quantitative, so that when I see something, I can make some judgment about this, whether it is something that I should be concerned about or do. On a couple of occasions I have called Ron Ney and said, "Okay, Ron, what does this mean?"

The answer is submit the thing and when it comes through and we review it, then we'll tell you what it means. That takes time. Time is a lot of money, not only from the standpoint of the work we'd be continuing to do on that project, but because there may be other priorities to which you'd like to switch. Time is money being spent to continue a given project for another year or year and a half before you get a readout from the reviewers at EPA as to what it means. I would like to be in a position to be able to make some assessments of my own, more than just gut feelings.

COMMENT: I think in Cleve Goring's book there is an environmental profile that lists water solubility, volatility, toxicity to quail, and a number of other factors. There are little bars, and it's all listed in terms of either a concentration or an LD_{50}. It's always a concentration, and you can draw a little stream going down through there and if you're in the stream, you're pretty safe through all of these factors. If you have one or more of these that fall outside of that little directional line, then you should start querying the thing. These relatively simple factors which you do early in the game are less expensive than a two-year carcinogenic study. But you really would like to know what it means, like in simple octanol/water.

MS. SCHUTH: What you're asking is what are the criteria on which decisions are based? That's obviously the job of the Criteria and Evaluation Division and they have written some of the criteria and are continually involved in this process. For example, they've written criteria which involve soil persistence. That's what you're asking for and that's what you're getting. It takes a lot of time and it's a big job.

Bioaccumulation is one of those concerns which is a current item for criteria development at the present time. I think as an industrial representative it is certainly within your province to push on the Registration Division people and ask, "What are the criteria that you're using?" They can in turn push on the Criteria and Evaluation people to define philosophy within which your results will be interpreted. Even if they wanted to give you a number, I don't think you want a number because you have to evaluate each chemical according to its use as well as other things.

Those criteria are forthcoming. I agree it would be nice to have them all now. The Criteria and Evaluation Division has had a lot of other jobs to do too and these criteria maybe haven't received as much attention as they deserve. But push for it, just say to the Agency, "It's registration criteria that we need. These delays are keeping our products off the market."

COMMENT: All I'm saying is there should be a mechanism and hopefully it will be forthcoming, partly through the benchmark chemistry program, partly through other things, where you can draw profiles of things, something where we can at an early stage in the game assess what we're going to do with the chemical, not just from the process standpoint but from all these things.

COMMENT: That's one of the reasons for the microcosm approach. The idea is that it may not tell you when you've got a completely safe compound. There may be things that you would overlook. You would tend to err in that direction. The theory is that you're going to detect any significant problems early, using some kind of integrative mechanism like a vole or whatever the organism might be that's in your microcosm. That tells you that you better do a little more extensive testing on that material before you go too much further with it.

QUESTION: Since I don't really work in the system, I'm not really aware of the disadvantages of the system, or as Dr. Buckley had said, what is it telling you and what is it not telling you? Is it possible that by trying to integrate all these species all at once, and then treating the whole system with the pesticide, you've totally distorted the thing? Are we distorting the system because you're exposing everything all at once to it and that doesn't really happen always in the environment?

COMMENT: The answer is very definitely yes, we're distorting it, for the same reason that we do high chronic dosage studies and 2-year feeding trials to try to accelerate toxic advantage, or in drug testing do accelerated 90-day feeding studies, logarithmic increases of doses and so forth. We have to reach some sort of conclusion quickly. Like you say, time is money. We're accentuating the biotic elements in terms of population densities, in terms of interactions. The processes which we're trying to represent in the environment are yet there. Predation, for example, is a process. Soil binding is a process. Leaching is a process. Volatilization is a process. We're including these processes in the systems. This is the reason that Allan has a very complex, physical structuring because he doesn't want predation at certain stages of development, but he does want predation of the adults.

He has to construct his flow-through system to get a certain kind of biological process simulated within the laboratory system. We have to have intensity of this process at a rate that we can measure. If we had absolutely perfect simulation counters and no quenching and good carryover of all of our material, if we could get the technical material labeled in the same way that we can get the pure quail to put in our chambers, we would improve the sensitivity of our analysis in terms of the one percent or the 10th of a percent impurity.

I would like to see us be able to do that. I would say from a microcosmic experiment, one could predict very accurately whether or not one should run extensive leaching studies or a very simple, short-term leaching study. If you have 65 percent loss of dieldrin in 30 days or 20 days, I would suggest that you do a lot more photochemistry and a lot more soil loss and a lot more loss from glass plates studies than you might want to do for a compound for which you had 10 percent loss in the total system or 10 percent unaccountability in your total system in 90 days or 60 days.

If we can increase the intensity of the interactions, particularly the biological interactions which is where the action is in terms of ecosystems effects, then we can come up with better rate constants. Maybe these are not equivalent to the rate constants of nitrification or ammonification in the environment, or of predator-prey relationship, but they are representative on a comparative basis. We use dieldrin as a standard, they use DDT as a standard, Pete can use mirex. We can standardize it against a reference chemical, and as long as we understand what the between system meaning is, then we can make some judgment as to what sort of questions we want to investigate in a much more sophisticated, detailed way, using a specialist in that area.

I'm not a photochemist and I haven't worked in photolysis for 13 years, and yet I know that if we look at the TLC profile of a microsomooxydase-treated preparation, or of a glass plate-photolyzed preparation, we're going to see similar qualitative, metabolic patterns, and we know where to go from there if we really want to know what those chemicals are. If you have a chemical that looks good in the field and in other applications, you're going to follow it out that far. I've seen the work that you do and you follow it down the line until you find no toxicity in your products.

COMMENT: I'd follow that up quickly. My point was that the whole system -- all of the vertebrates, all of the invertebrates, the whole works -- is totally exposed to the maximum level of the pesticide all at once, whereas that may not really be the situation. You said, yes, we have to stress the situation in order to get an answer out of it. Are the numbers, whatever is generated from each individual species, weight averaged? In other words, I've seen the results from Metcalf's study on our one compound, and I see the numbers for all of the species. They are submitted, and the problem is what weight do you place upon each species now? It may be so much farther down the food chain, although it has a high number, that it would have never gotten to that point.

My point about an artificial situation is that maybe it's in the mechanics of dealing with the numbers that you get from there to get the final index again, but that it has to be weight averaged in order to really look, because something that you say is bad for that one small microorganism may never get there.

COMMENT: In any kind of modeling context, whether it's mathematical or an econometric model, for example, on locating an industry, there are all kinds of simplifications built into that sort of thing, and I don't see how you can avoid that. Models are to give you general ideas or general indications of whether you've got a problem or not.

QUESTION: Is this one aspect of half a dozen different systems that exist when comparisons are made between different systems? Will it ever get to a point where one system is considered to be the best and everybody follows that as a standard, or will there be several systems that get to be best?

COMMENT: That will happen.

QUESTION: Weight averaging might be an inclusion in the interpretation of the data. That will be one point. We talked about interrelations this morning of different factors and mathematical model. These factors were not spelled out exactly, but I got the impression they were exclusively scientific points of data. However, there are other social and economic factors that are actually related to those scientific points. In other words, how many millions of acres of corn are planted or destroyed? How many people does that wind up feeding? What is the starvation rate? How many jobs does that affect? How many kids are born? It can go on and on. Those are points which maybe we as scientists say, "That's not for me to judge," but they have to be included somewhere.

COMMENT: That concept is really the general case of how you approach a model from a topological point of view, and the model could include socioeconomic characteristics.

COMMENT: That I didn't know.

COMMENT: Production.

COMMENT: That's what I wanted to know.

COMMENT: Any number of features. The method itself can be generalized to a model of any degree of complexity.